# NIDDK

## Recent Advances & Emerging Opportunities

February 2013

U.S. Department of Health and Human Services
National Institutes of Health
National Institute of Diabetes & Digestive & Kidney Diseases

**The cover design is adapted from a poster advertising a seminar entitled "Space, Place, and Environment in Obesity Research: Geospatial Approaches," which was held in April 2012 and sponsored by the NIH Obesity Research Task Force. The Task Force, for which NIDDK Director Dr. Griffin Rodgers serves as Co-chair, was established in 2003 to accelerate progress in obesity research across the NIH. (Please see the Obesity Chapter for more information about NIDDK's obesity research portfolio.)**

# NIDDK

# Recent Advances & Emerging Opportunities

February 2013


U.S. Department of Health and Human Services
National Institutes of Health
National Institute of Diabetes & Digestive & Kidney Diseases

## CONTENTS

# Message from the Director

As the Director of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), I am pleased to present this annual compendium highlighting the research efforts and programs supported by the Institute. The NIDDK has a broad research responsibility that includes some of the most common, debilitating, and costly conditions affecting Americans. These conditions include diabetes and other endocrine and metabolic diseases, such as cystic fibrosis; liver disease and other digestive diseases, such as inflammatory bowel disease; nutritional disorders and obesity; kidney diseases, such as polycystic kidney disease; urologic diseases and conditions, such as interstitial cystitis/ painful bladder syndrome; and hematologic diseases.

The 13th edition of this report illustrates recent NIDDK-supported scientific advances, such as:

- Identification of genetic variants associated with elevated risk for type 2 diabetes in African American and South Asian populations
- Demonstration that controlling blood glucose early in the course of type 1 diabetes preserves kidney function for decades
- Findings from a clinical trial that lifestyle changes leading to weight loss and increased physical fitness slow the decline in mobility in overweight or obese adults with type 2 diabetes
- Discovery that the composition of bacterial species that populate the human gut evolves with age and differs among people from diverse geographic regions
- Identification of cellular factors that induce fat-storing white adipose tissue to take on properties of calorie-burning brown adipose tissue
- Finding that obesity and high-fat diet are associated with damage to an area of the brain that regulates body weight
- New insights that illuminate the complex system of regulation surrounding kidney fibrosis following injury
- Findings from a clinical trial that invasive and costly tests commonly performed in women before surgery for stress urinary incontinence may not be necessary in many cases
- Demonstration that nutritional supplementation with an essential amino acid improves the anemia and developmental defects associated with Diamond-Blackfan anemia in animal models

This report also includes personal stories of patients. The mother of a child with type 1 diabetes explains the daily effort required to manage the disease and her family's dedication to participating in research to combat it. A college student shares his experience participating in a clinical trial to test treatment options for type 2 diabetes in youth. A woman describes the challenges of living with cystic fibrosis and her experience participating in a clinical trial testing treatments for cystic fibrosis-related diabetes. A woman donates a portion of her liver to her friend with chronic liver disease; both share their perspectives on this gift of life. A woman with interstitial cystitis/painful bladder syndrome and irritable bowel syndrome shares her experience participating in a research network to understand the underlying causes of urological chronic pelvic pain syndromes.

The NIDDK is continuing efforts to ensure that knowledge gained from its research advances is disseminated to health care providers, patients, and the general public. Such efforts include the Institute's education programs: the National Diabetes Education Program and the National Kidney Disease Education Program. Additionally, the Weight-control Information Network, the National Diabetes Information Clearinghouse, the National Digestive Diseases Information Clearinghouse, and the National Kidney and Urologic Diseases Information Clearinghouse develop and distribute science-based information on diseases and disorders within the NIDDK mission. Several hundred brochures, fact sheets, and publications are available in printed format and on the NIDDK web-site so that they are readily available for patients, health care providers, and the public. I invite you to visit the web-site at *www.niddk.nih.gov*

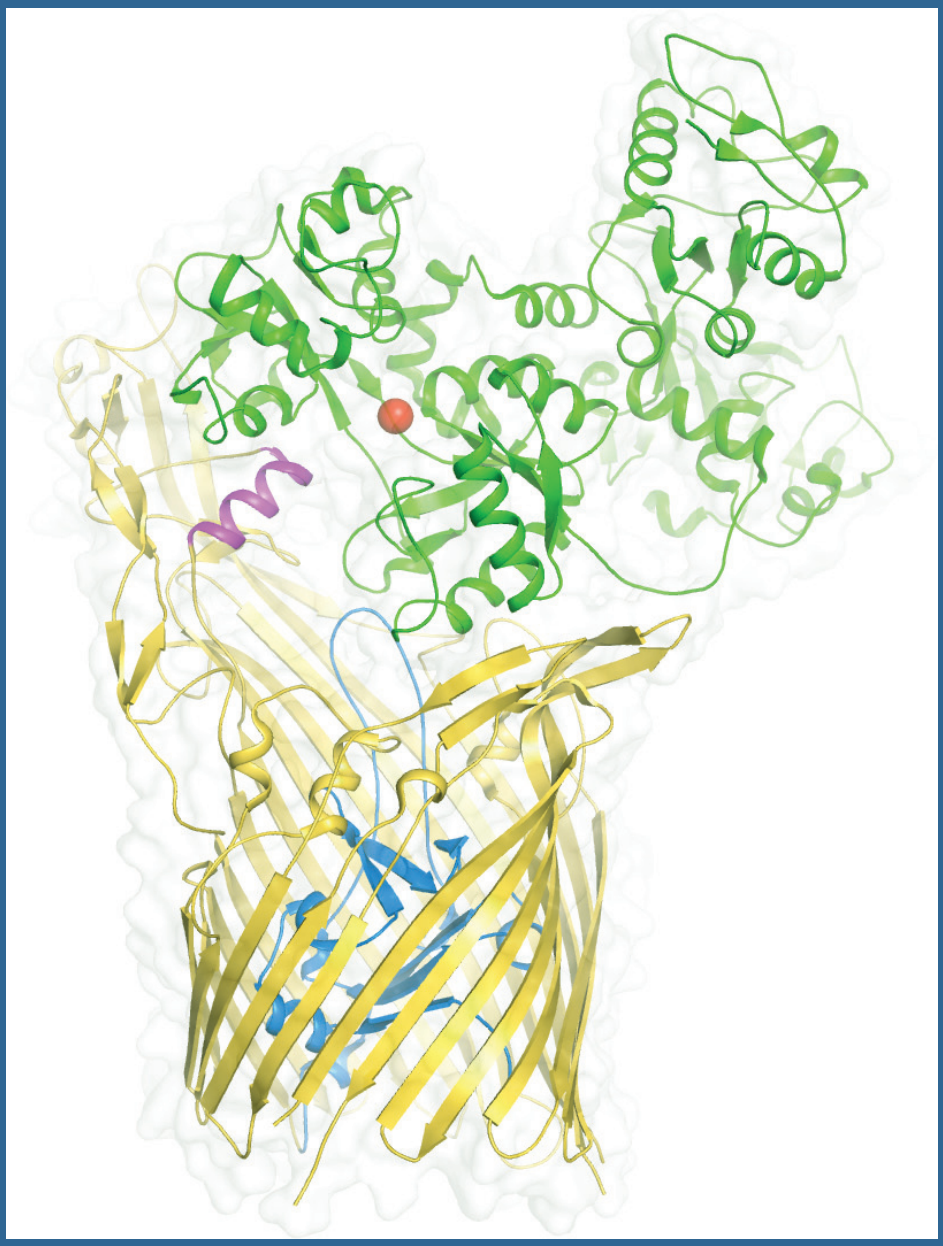
The efforts featured in this publication reflect the core mission of the NIDDK, including the Director's guiding principles:

- Maintain a vigorous investigator-initiated research portfolio
- Support pivotal clinical studies and trials
- Preserve a stable pool of talented new investigators
- Foster exceptional research training and mentoring opportunities
- Ensure knowledge dissemination through outreach and communications

This report reflects only a fraction of the immense body of NIDDK-funded research performed by basic scientists, clinical investigators, and patient volunteers. Moving forward, we remain committed to supporting these important areas of research and translating scientific discoveries into improvements in the health and quality of life of all people.

**Griffin P. Rodgers, M.D., M.A.C.P.**
Director
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
U.S. Department of Health and Human Services

Scan the QR code with your mobile device to link to the NIDDK web-site at *www.niddk.nih.gov*



The bacteria *Neisseria* can cause serious diseases in humans. These disease-causing bacteria require iron for their survival and use a surface receptor to extract iron directly from a human iron-binding protein abundant in the bloodstream. Shown here is the structure of the bacterial receptor protein (shown in gold, blue and magenta) extracting an iron atom (red sphere) from the human protein (green). Details about molecular interactions between complex proteins may identify new targets for treatments.

*Image courtesy of Dr. Nicholas Noinaj, NIDDK.*

# Cross-Cutting Science

*Advances in medicine sometimes are achieved in great leaps, but more often result from the gradual accumulation of new knowledge over years. Insights into fundamental biologic processes at the smallest levels of an organism—its genes, the proteins they encode, the inner workings of cells, and the ways cells communicate with each other—can have broad and far-reaching implications. Indeed, many significant advances in our understanding of disease and in the development of novel treatments can be traced to laboratory studies whose immediate relevance to health could not have been fully known or appreciated at the time they were conducted. With the development of innovative technologies, novel scientific approaches, and the emergence of new scientific disciplines as teams of talented, creative researchers join together to pursue increasingly complex challenges, new opportunities to make exciting discoveries arise each day. Described in this chapter are several recent studies, whose themes span multiple areas within the NIDDK research mission. Also featured are the NIDDK efforts at public outreach to better disseminate important health information to the public using both traditional and social media.*

*The efforts outlined here illustrate the Institute's commitment to basic and applied research that is relevant across a broad spectrum of science. The insights gained through this research can be expected to aid progress in many scientific endeavors, for today's discoveries may hold the seeds of tomorrow's cures.*

## NEW INSIGHTS INTO STEM CELLS

**Induced Pluripotent Stem Cells Hold on to Past Identity:** Researchers have found genomic marks in human induced pluripotent stem (iPS) cells that currently limit their scientific and therapeutic potential, but also suggest opportunities to improve the development of these cells.

Researchers initially developed iPS cells with the hope of overcoming challenges posed by other types of stem cells. Human embryonic stem (ES) cells, for example, hold promise in the treatment of disease because they are "pluripotent," meaning that unlike most other cells, they have the ability to form virtually any cell type and thus could generate cells for repair of human tissues and organs. The use of human ES cells, however, is controversial because their isolation entails the destruction of early-stage human embryos; ES cells have other limitations as well. In recent years, scientists developed strategies to reprogram cells, such as blood or skin cells, to revert from their specific cell types back to an ES cell-like state, with the potential to form not only new cells of their original type, but also stem cells and a multitude of different cell types. These pluripotent, reprogrammed cells, called iPS cells, could potentially be used to study diseases and to generate cells to treat specific diseases, potentially with a tissue match for the recipient (avoiding transplant rejection).

For reasons that have been poorly understood, iPS cells generated to date are significantly less pluripotent than ES cells; they are more easily able to form the cell type from which they were originally derived than to form cells of other types. To understand why this might be the case in human iPS cells, researchers analyzed the cells' DNA, building on previous findings in mouse cells. Mouse iPS cells retain a pattern of chemical modifications on their DNA characteristic of their past cell type, rather than a pattern characteristic of ES cells. Although this modification does not alter the sequence of the genetic code, it can affect the cell's ability to turn genes on or off. The combination of genes that are active and inactive characterizes a cell type; therefore

this important modification has the effect of helping a cell to "remember" its identity. In this new research, scientists sought to determine whether human iPS cells retained the chemical modifications of their past cell type, like the mouse iPS cells did. They produced iPS cells from both blood and skin cells. The researchers found, as expected, that blood-derived iPS cells were more likely to form blood cells and skin-derived iPS cells were more likely to become skin cells. By comparing the patterns of chemical modifications of iPS cells to ES cells, the researchers determined that the iPS cells retained patterns characteristic of their original cell types. Current techniques to generate iPS cells, therefore, do not fully erase the cell's memory, limiting its potential to become another cell type.

Scientists will continue research to develop new techniques that may be able to erase the residual patterns more fully. In the meantime, scientists may be able to take advantage of the bias of iPS cells toward their original cell type in the study of and development of therapies for diseases associated with those cell types. Cautious optimism continues for the eventual, wider use of iPS cells.

*Kim K, Zhao R, Doi A, et al. Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. Nat Biotech 29: 1117-1119, 2011.*

**Genetic Mutations in Experimentally Derived (Induced Pluripotent) Stem Cells:** Researchers have recently determined the source of genetic mutations found in induced pluripotent stem (iPS) cells. In recent years, scientists have developed ways of reprogramming cells, such as those derived from blood or skin, to revert back to an embryonic stem cell-like state. These stem cells have the potential to give rise not only to new cells of the original type but also to more stem cells and to cells of many different types of tissues. One technique involves the introduction of three to four genes into adult cells that direct the reprogramming of these cells into iPS cells. Recent studies assessing the genetic integrity of iPS cells have found mutations that could limit the therapeutic potential of these cells, but the origins of these mutations have not been clear.

Scientists determined the complete sequence of all the DNA—the "genome"—from 10 different mouse iPS cell lines derived from three different original, or parental, cells. The entire genomes were sequenced to determine the number and location of any mutations. Hundreds of mutations were detected in each iPS cell line genome. By comparing the genome sequences of the parental cells to those of derivative iPS cells, the researchers concluded that most of the genetic mutations in iPS cell lines were not caused during the reprogramming process but rather were derived from mutations that pre-existed in the parental cells.

This study illustrates the importance of selecting the most appropriate adult cell to undergo the reprogramming process to form iPS cells, because any mutations that exist in the parental cell will be passed on to the iPS cells. Knowledge gained from sequencing iPS cell genomes may help to improve the selection and derivation of iPS cells such that these cells can be more safely used for regenerative therapies.

*Young MA, Larson DE, Sun C-W, et al. Background mutations in parental cells account for most of the genetic heterogeneity of induced pluripotent stem cells. Cell Stem Cell 10: 570-582, 2012.*

**Aging of Blood Stem Cells:** Recent research has linked blood stem cell aging with increased activity of the cell division cycle 42 (Cdc42) protein. All blood cell types are derived from a population of self-renewing hematopoietic (blood) stem cells (HSCs) that first appear during embryonic development. However, there are changes in HSC structure and a decline in function over time that leads to reduced production of both red and white blood cells by the HSC. The molecular mechanisms underlying HSC aging are not known, but previous research has shown that Cdc42 activity is significantly increased in both HSCs and other cell types of old mice when compared with those from young mice. Whether enhanced activity of Cdc42 is responsible, in part, for the aging phenotype is not known.

Using a mouse model that exhibits increased Cdc42 activity due to genetic deletion of an inhibitor of the

protein, scientists found that HSCs from young animals with this mutation exhibit a decline in function similar to that observed in normal older animals. HSCs from young mutant mice showed diminished organization of intracellular structures similar to that seen in aged mice. HSCs from the mutant mice that were grown in culture in the presence of a drug that inhibits Cdc42 activity reverted to a "younger" appearance, with intracellular organization restored. This observation provides evidence that Cdc42 activity plays a role in structural degradation seen in the aging of HSC, and that this can be reversed through inhibition of this protein.

Additional studies suggested that Cdc42 plays a role in the decline in HSC function observed during aging. Using an experiment that measures how well HSCs are able to "home" or localize to the bone marrow and begin to grow there, the scientists found that HSCs from a mutant mouse with elevated Cdc42 activity no longer had the ability to repopulate the hematopoietic system of a mouse whose HSCs have been eliminated by prior radiation treatment. In contrast, cells from a mutant mouse that had been treated with a Cdc42 inhibitor in culture prior to being injected into the recipient mouse had an increased ability to repopulate the bone marrow, though not as robustly as normal HSCs from young animals. This finding shows that even a brief exposure to the Cdc42 inhibitor was sufficient to partially restore function lost during aging of HSCs.

Future studies will determine whether drugs that target Cdc42 can restore function in aged human HSCs. As elevated Cdc42 activity has been observed in multiple tissues in aged mice, this finding may have implications that extend beyond the hematopoietic system and blood cell production.

*Florian MC, Dörr K, Niebel A, et al. Cdc42 activity regulates hematopoietic stem cell aging and rejuvenation. Cell Stem Cell 10: 520-530, 2012.*

### Studies Shed Light on Resident Stem Cells that Repopulate Normal and Injured Intestine:

Two research teams have illuminated unique roles in intestinal regeneration for two distinct stem cell populations found on the inner surface of the intestine. The inner lining of the intestine plays an essential role in absorption of nutrients and balancing absorption and secretion of water and electrolytes, as well as providing a barrier against entry of bacteria. The lining is only one cell thick and cells slough off the surface continuously, lasting only about 1 week, requiring a process of continuous regeneration. Cells are also replaced following any injury. Recently, two populations of intestinal stem cells that are necessary for regeneration were identified by their different locations in the intestinal surface, as well as the unique proteins marking their outer membranes—either Lgr5 or Bmi1. Two research teams took a closer look at these stem cells to characterize their roles in intestinal regeneration.

One of the teams conducted research as part of the NIDDK's Intestinal Stem Cell Consortium. Using a microscopic technique that highlights intestinal stem cells with fluorescent markers for Lgr5 and Bmi1 in genetically modified mice, the researchers measured proliferation of the cells under normal, healthy conditions, as well as after injury caused by radiation. They found that the stem cells with Lgr5 on their surface actively proliferated under normal conditions but were destroyed by radiation. The stem cells marked by Bmi1 were relatively inactive under normal conditions but resisted radiation and proliferated dramatically following the injury. The Bmi1-marked stem cells in culture could also form some Lgr5-marked stem cells, showing their capacity to repopulate the intestine with both stem cell populations following injury.

Another team examined whether the Paneth cell, another type of intestinal cell located near Lgr5 stem cells, was essential for the ability of Lgr5 stem cells to repopulate the intestinal lining as part of normal cell turnover and tissue renewal. Paneth cells are known for secreting substances, such as proteins with antimicrobial properties, enzymes, and growth factors. Considering their proximity to intestinal stem cells, Paneth cells were thought to be involved in stem cell functions. This study used genetically modified mice and fluorescent markers to identify the Lgr5-producing stem cells and Paneth cells during early intestinal development. They found that the appearance of the intestinal stem cells preceded

Paneth cells in the developmental process, and that the stem cells appeared to function normally. They then created a genetically modified mouse that lacked Paneth cells. The stem cells functioned normally in this mouse model as well, proliferating to renew the intestinal surface as usual. These experiments indicate that Paneth cells are not essential for many aspects of Lgr5-marked stem cell function, though Paneth cells may still have other beneficial effects on their neighboring stem cells.

Taken together, the work of these two research teams paints a more nuanced picture of the complementary stem cell types that renew the intestinal surface throughout life, in terms of continuing cell turnover as well as regeneration following injury. This knowledge could be applied to optimizing recovery from different forms of intestinal injury.

*Kim T-H, Escudero S, and Shivdasani RA. Intact function of Lgr5 receptor-expressing intestinal stem cells in the absence of Paneth cells. Proc Natl Acad Sci USA 109: 3932-3937, 2012.*

*Yan KS, Chia LA, Li X, et al. The intestinal stem cell markers Bmi1 and Lgr5 identify two functionally distinct populations. Proc Natl Acad Sci USA 109: 466-471, 2012.*

## PROTECTING THE GENOME AND REGULATING GENE EXPRESSION

**Long-range Regulation of How Genes Are Turned "Off" and "On":** Two studies have advanced our understanding of the transcriptional regulation of a set of red blood cell genes call globin genes, and of how these genes are turned on and off. Transcription is a biologic process that involves the transcribing or copying of genetic information from DNA into RNA. Immature red blood cells produce globin proteins, key components of hemoglobin, which carry oxygen in red blood cells from the lungs to the rest of the body. Production of globin proteins is a highly regulated process to ensure that these genes are turned on ("expressed") or off at appropriate times during the development of red blood cells from their precursors in the bone marrow.

The mammalian *β-globin* gene locus was among the first gene clusters to provide insight into how gene regulation is influenced by long-range chromosomal interactions between DNA sequences far from and near to the protein-coding segment of a gene. Specifically, these interactions occur between a powerful element called an enhancer that helps turn on the *β-globin* gene, also referred to as the gene's locus control region (LCR), and a DNA element called a promoter, which is immediately adjacent to the gene and helps regulate whether it is on or off. Scientists continue to study enhancers such as the LCR in order to more fully understand their role in the regulation of gene expression generally.

In the first study, scientists devised a strategy to delineate whether loops of chromosomal DNA, created by the interaction between the *β-globin* gene promoter and LCR, are a cause or an effect of gene transcription. Using erythroid precursor cells (cells that develop into normal red blood cells) to study the *β-globin* gene locus transcription process, researchers showed for the first time that the protein Ldb1 is a key looping factor involved in long-range regulation of gene transcription. Furthermore, their experimental manipulations that forced or impaired the creation of the DNA loop which allows the LCR to interact with the *β-globin* gene confirmed that loop formation plays an important role in initiating *β-globin* gene transcription and is not simply a consequence of the gene being "turned on."

In the second study, investigators sought to determine the relative contribution of four segments of the LCR to its function in regulating *β-globin* gene transcription and also to determine the location of the *β-globin* locus within the cell's nucleus. Nuclear location influences whether or not a gene is expressed. Scientists refer to these LCR segments as DNase I hypersensitive sites (HSs) because they are regions of the enhancer that are extremely sensitive to breakdown into smaller pieces (digestion) by the enzyme DNase I. Sensitivity to DNase I is a measure of the transcriptional status of a gene and indicates that this region of DNA is "open" or exposed, so that factors that promote transcription can bind to it. Mice were engineered that had various combinations of HSs deleted, and the effects of these

deletions on gene transcription were measured in relationship to the position of the *β-globin* gene locus in the nucleus. The results showed that while the effects of the HSs are additive, only two were needed for the *β-globin* locus to be repositioned towards the center of the nucleus where gene transcription can become active. However, all four of the HSs of the LCR were shown to be needed for *β-globin* transcription to be completed efficiently.

These studies add considerable knowledge to our understanding of the regulation of gene transcription. Identification of looping factors such as Ldb1 and delineation of the various components required for proper *β-globin* transcription may help the development of new ways to treat hematologic diseases, such as sickle cell disease, by reactivating dormant hemoglobin genes.

*Bender MA, Ragoczy T, Lee J, et al. The hypersensitive sites of the murine β-globin locus control region act independently to affect nuclear localization and transcriptional elongation. Blood 119: 3820-3827, 2012.*

*Deng W, Lee J, Wang H, et al. Controlling long-range genomic interactions at a native locus by targeted tethering of a looping factor. Cell 149: 1233-1244, 2012.*

**Guiding Genetic Rearrangement To Protect the Genome:** Scientists discovered that genetic rearrangement—the process by which cells intentionally break, shuffle, and repair their DNA to create new combinations of genes—is directed away from functional genomic regions. Genetic rearrangement occurs naturally in cells destined to become sperm and eggs to generate genetic diversity, ensuring that each new organism will be unique. This process can be beneficial in that new, advantageous traits can arise from these genetic rearrangements; but, if it goes awry, this process can also generate abnormalities that result in miscarriages, congenital birth defects, and mental retardation. Researchers in the NIDDK's Intramural Research Program and colleagues previously demonstrated that locations in the genome where genetic rearrangements occur more frequently—"hotspots"—were associated with the activity of a protein called PRDM9, but they did not know if the hotspot location was determined by PRDM9.

To evaluate PRDM9's role, the researchers mapped hotspots in two mouse strains that were nearly genetically identical, but encoded different versions of PRDM9. They found a similar number of hotspots in the two different strains, but almost no overlap in the locations of the hotspots. Hotspots in the two lines centered on distinct DNA sequences that aligned with the predicted DNA-binding sites for the different PRDM9 proteins. This indicated that the location of rearrangement hotspots was dependent on PRDM9. When the scientists looked at mice genetically engineered to lack PRDM9, they found that rearrangements still occurred in hotspots, but that these hotspots were not in the same locations as those in normal mice. Rather, the hotspots in mice without PRDM9 were re-routed to sites in the genome associated with gene activity. The researchers propose that PRDM9 directs the rearrangement machinery to preferred sites in the genome and away from functional genomic regions. This re-routing away from important genomic elements may protect against potential harmful effects of genetic rearrangement.

*Brick K, Smagulova F, Khil P, Camerini-Otero RD, and Petukhova GV. Genetic recombination is directed away from functional genomic elements in mice. Nature 485: 642-645, 2012.*

**Multi-tasking UTX Protein Plays Key Role Early in Development:** NIDDK intramural scientists studying mice have discovered that a protein capable of unleashing genes important to tissue formation has a second, independent job regulating early embryonic development. As a fertilized egg develops into an embryo, it must first grow and divide to organize into a ball of cells containing three distinct cellular layers, called germ layers. Each germ layer will go on to generate a specific subset of the organs, tissues, and cells that make up an organism. Embryonic stem (ES) cells grown in the laboratory can be coaxed to differentiate into the three germ layers, thus offering a model to understand factors influencing this critical

aspect of early development. The UTX protein has already been shown to regulate processes important later in development, such as those governing generation of the heart and muscle cells. In some cases, this occurs when UTX exerts an enzymatic function that enables quiescent developmental genes to become activated. Now, researchers studying UTX in mouse ES cells and mouse embryos have discovered that this protein is also critical to an early step in development—formation of one of the three germ layers—and that this function is independent of its enzymatic activity.

Working in mouse ES cells, the researchers generated three experimental strains: a control strain with an intact *UTX* gene, a strain in which the *UTX* gene was deleted, and a strain in which the *UTX* gene was intact but mutated so that the protein produced no longer had enzymatic activity. These different strains were treated to encourage formation of germ layers. While apparently able to support formation of other germ layers, ES cells lacking UTX could not form a layer called the mesoderm. In contrast, ES cells with the mutated UTX developed similarly to the control cells—indicating that UTX, but not its enzymatic activity, was important to mesoderm formation. Further experiments revealed a possible mechanism: UTX protein binds to a DNA region controlling activation of the gene for Brachyury, a factor essential to mesoderm formation. In the absence of UTX, *Brachyury* gene activation in ES cells was reduced significantly, and could not be artificially induced—indicating that UTX is an essential component of the molecular machinery that activates *Brachyury*.

The *UTX* gene is found on the X chromosome and is thus normally present in both male and female mice. Male cells possess a gene on the Y chromosome, called *UTY*, that encodes a protein similar to UTX but which lacks detectable enzymatic activity. Suspecting that UTY may act like UTX, the researchers conducted a series of molecular experiments and found evidence suggesting that UTY can also activate *Brachyury*. To test whether UTX and UTY are important in actual embryonic development, the researchers mated mice to produce female embryos lacking UTX, and male embryos lacking UTX but retaining UTY. Female embryos lacking UTX had significantly reduced *Brachyury* activation, severe developmental defects similar to those seen in mouse embryos lacking the *Brachyury* gene, and died before birth. In contrast, male embryos lacking UTX, while suffering defects that made it impossible for them to survive much beyond birth, developed much more normally and activated *Brachyury*—further suggesting that UTX and UTY are functionally redundant early in development. Together, these findings shed further light on both early development and the multi-tasking of factors during development—information that could also be useful in recapitulating developmental processes important to regenerative medicine efforts.

*Wang C, Lee J-E, Cho Y-W, et al. UTX regulates mesoderm differentiation of embryonic stem cells independent of H3K27 demethylase activity. Proc Natl Acad Sci USA 109: 15324-15329, 2012.*

## BASIC SCIENCE RESEARCH REVEALS NEW INFORMATION ABOUT CELLULAR PROCESSES AND METABOLISM

**Biological Chemistry: How Is a Rare Bond Created?** An ancient enzyme that can be traced back 500 million years forms the chemical "rivet" that reinforces connective tissue throughout the body. This "sulfilimine" chemical bond links a nitrogen and sulfur atom in a manner that had not been observed in a biological system until 3 years ago. The sulfilimine bond reinforces the collagen IV network, which in the kidney, provides the structural scaffolding for the glomerular basement membrane. Researchers explored the chemistry underlying the creation of this rare bond and found that it is created by the enzyme peroxidasin.

The researchers used cultured mouse cells to study the how the sulfilimine bond that links one collagen segment to another is created. They characterized each of the components and steps involved in the formation of this bond by peroxidasin, which include the generation of a highly reactive and potentially toxic intermediate acid that is similar to household bleach. The elucidation of the process that leads to the creation of this rare chemical bond is an important advance in

researchers' knowledge of collagen biochemistry at a fundamental level and provides broader insights into one way that these networks are stabilized.

Levels of peroxidasin are increased in models of high blood pressure and atherosclerosis, suggesting that it may play a role in inflammation and disease. The researchers hypothesize that these findings could also provide insight into treatment of the autoimmune disease Goodpasture's syndrome, in which antibodies target collagen IV molecules. Future studies may elucidate additional roles of this enzyme in a variety of diseases and illuminate possible approaches to treatment.

*Bhave G, Cummings CF, Vanacore RM, et al. Peroxidasin forms sulfilimine chemical bonds using hypohalous acids in tissue genesis. Nat Chem Biol 8: 784-790, 2012.*

**Bacterial Strategy To Acquire Iron from Humans:** A three-dimensional reconstruction detailing the interactions between proteins from the bacteria *Neisseria meningitidis* and the human iron-binding protein transferrin has recently been determined. Two members of the *Neisseria* family of bacteria can cause disease in humans; one of these, *N. meningitidis*, is a leading cause of meningitis. It requires iron both for its survival and to cause disease. *N. meningitidis* collects iron by extracting it from the human transferrin receptor, a protein on the cell surface that binds to iron in the blood and transports it into cells.

To acquire iron, the bacterium uses two proteins on its surface, TbpA and TbpB, that can bind to and remove iron from the transferrin receptor in the human host. The exact nature of this interaction is unknown. Determining the structure of these bacterial proteins and how they interact with host proteins is an important step in understanding how they function.

Researchers have, for the first time in the laboratory, generated complexes of TbpA and TbpB bound to human transferrin that permitted the assembly of a three-dimensional reconstruction defining important interaction sites between the bacterial proteins and transferrin. They examined complexes of TbpA and transferrin, TbpB and transferrin, and of all three proteins together using three different methods. The reconstruction suggests a mechanism for bacterial uptake of iron, including how the bacteria latches on to transferrin, how a part of protein TbpA inserts itself into and displaces the iron from transferrin, and how the recently acquired iron is transported inside the bacteria.

This study provides critical information regarding the structural basis for iron piracy by disease-causing bacteria. Furthermore, as TbpA and TbpB are on the surface of the bacteria, this finding may have implications for the development of both structure-based vaccines and drug design.

*Noinaj N, Easley NC, Oke M, et al. Structural basis for iron piracy by pathogenic Neisseria. Nature 483: 53-58, 2012.*

## A NOVEL APPROACH TO ALLEVIATE CHRONIC PAIN

**New Insights into Chronic Pain:** Researchers have shown that molecules that activate the $A_3$ subtype of the cellular receptor for the molecule adenosine have a potent anti-pain effect in two different rodent models of chronic pain. These results identify a novel class of drugs that could be used to alleviate chronic pain arising from various causes, including some forms of chemotherapy for cancer.

"Neuropathic pain" is caused by damage to the nervous system. It often manifests as abnormal sensations, such as numbness or tingling, or as pain produced by mild stimuli that are normally not painful, such as light touching. Using a mouse model of pain sensation, the scientists observed that treatment with any one of three different molecules that selectively activate the $A_3$ subtype of the adenosine receptor—receptor "agonists"—could alleviate neuropathic pain. Similar pain relief following treatment with $A_3$ adenosine receptor agonists was seen in rats that had been given drugs used in chemotherapy. While these agents were effective in alleviating neuropathic pain, they had no effect on so-called "nociceptive pain," which is pain sensed in response to a potentially harmful stimulus such as heat.

Notably, the administration of any one of the three $A_3$ receptor agonists at very low doses could significantly increase the effectiveness of opiate pain relievers, such as morphine, in the mouse model. Studies of cells in culture showed that the $A_3$ receptor agonists did not limit the ability of chemotherapeutic drugs to inhibit the growth of cancer cells, an important consideration given that patients undergoing chemotherapy sometimes develop neuropathic pain that is so debilitating that it leads them to discontinue treatment.

Currently, the most effective treatments for chronic neuropathic pain involve opiate-derived drugs. However, their usefulness is limited because patients often develop tolerance for the drugs, necessitating higher and higher doses to achieve pain relief, and because these high doses can result in serious side effects, including the possibility of addiction. The identification of the $A_3$ adenosine receptor signaling pathway as a potential target for treatment of chronic neuropathic pain, and the existence of highly specific, potent activators of this pathway, represents a novel approach to treatment of chronic pain.

*Chen Z, Janes K, Chen C, et al. Controlling murine and rat chronic pain through $A_3$ adenosine receptor activation. FASEB J 26: 1855-1865, 2012.*

## INSIGHTS INTO HIV TRANSMISSION AND TREATMENT

### Drug Therapy To Prevent HIV Infection:

Researchers recently investigated whether two drugs that have been used to treat infection with HIV-1, the virus that causes AIDS, might also be used to prevent transmission of the virus. For years, a combination of drugs termed "highly active antiretroviral therapy" (HAART) has been used to treat people infected with the human immunodeficiency virus, HIV-1. The use of drugs that are part of HAART in individuals before they are exposed to HIV-1 has been considered as a possible strategy to prevent the transmission of this virus. Scientists have now examined the metabolism of two drugs used in HAART and have described their distribution in different tissues.

Because HIV-1 is often transmitted through sexual contact, it was important that the researchers accurately measure levels of the active forms of the drugs in genital and colorectal mucosal tissue. The investigators gave 15 healthy men and women a single oral dose of a combination of two antiretroviral drugs, tenofovir (TFV) disoproxil fumarate and emtricitabine (FTC), and subsequently measured the concentration of these drugs over the next 14 days in the volunteers' blood and genital secretions, as well as in their vaginal, cervical, and rectal tissues. The drugs were detected in the blood and genital secretions for the full 14-day duration of the study and were present at higher concentration in the genital secretions, with a particularly high concentration of FTC in these samples. The biologically active metabolites of the drugs were detected in the vaginal, cervical, and rectal tissues for varying durations and at different levels. The active form of TFV was found at high levels for all 14 days of the study in rectal tissue, but was present at much lower levels in vaginal and cervical tissue. The active form of FTC was present at higher levels in vaginal and cervical tissues than in colorectal tissues, but could be detected for less than 2 days.

The wide range of tissue exposure to an orally-administered drug reported in this study illustrates the need for more detailed studies of the pharmacology of drugs currently used to treat HIV infection as possible agents to prevent transmission of the virus. Ultimately, the success of drug therapy to prevent the spread of HIV-1 will depend on selecting the proper combination of drugs and their doses.

*Patterson KB, Prince HA, Kraft E, et al. Penetration of tenofovir and emtricitabine in mucosal tissues: implications for prevention of HIV-1 transmission. Sci Transl Med 3: 112re4, 2011.*

### Metformin Is Shown To Be Effective in Treating HIV Patients at Risk for Cardiovascular Disease (CVD):

In a recent clinical trial, scientists studying the effects of the drug metformin and lifestyle modification (LSM) on HIV-infected patients with metabolic syndrome found that metformin significantly reduced the progression of coronary artery calcification (CAC), a risk for CVD.

Lifestyle modification therapy was not as effective in achieving this goal. HIV-infected patients have high rates of metabolic abnormalities, including a large waistline, high levels of circulating triglycerides, low levels of HDL ("good") cholesterol, high blood pressure, and high fasting glucose. Having three or more of these contributing factors meets the criteria for metabolic syndrome and increases an individual's risk of developing CVD and type 2 diabetes.

Because metformin has been shown to significantly reduce CVD events in individuals without HIV, and because LSM is considered important in the treatment of HIV-infected patients with metabolic syndrome, clinical researchers hypothesized that treatment with metformin and/or LSM would be beneficial for HIV-metabolic syndrome patients in reducing CAC and other risk factors for CVD. To test this hypothesis, the researchers conducted a clinical study in which HIV-metabolic syndrome patients were divided into four groups and treated for 1 year with placebo alone, LSM and placebo, metformin alone, or metformin and LSM. Treatment with metformin and/or LSM was assessed by changes in several criteria including CAC, calcified plaque volume, and other measures of subclinical CVD. In addition, changes in several metabolic indices including fasting blood sugar and HDL-cholesterol levels were measured. Metformin was found to significantly reduce progression of CAC and calcified plaque volume in these patients and had a significantly greater effect on CAC progression than treatment with LSM. Also, metformin improved insulin resistance, a measure of prediabetes and type 2 diabetes. Although LSM had a lesser effect than metformin on CAC progression, it had significantly beneficial effects on HDL-cholesterol and cardio-respiratory fitness.

The results of this clinical study provide clinicians and researchers with valuable information on metformin as an effective treatment for preventing cardiovascular plaque progression in patients with HIV and metabolic syndrome. In addition, the study has shown LSM to be effective in treating some metabolic conditions in these patients. The metformin finding is particularly advantageous because, although additional studies are needed to assess whether metformin can prevent CVD events, metformin is an FDA-approved generic drug that could be used in the treatment of CVD risk factors in HIV-infected patients with metabolic syndrome.

*Fitch K, Abbara S, Lee H, et al. Effects of lifestyle modification and metformin on atherosclerotic indices among HIV-infected patients with the metabolic syndrome. AIDS 26: 587-597, 2012.*

# *Dr. Peter P. Reese and Dr. Georgios Skiniotis: NIDDK-Supported Scientists Receive Presidential Award*

Two scientists supported by the NIDDK have received the 2011 Presidential Early Career Award for Scientists and Engineers (PECASE). PECASE is awarded annually to scientists and engineers who, while early in their research careers, have demonstrated the pursuit of innovative research and outstanding scientific leadership. Among the recipients were two NIDDK extramural grantees—Peter P. Reese, M.D., M.S.C.E., and Georgios Skiniotis, Ph.D. In addition to the NIDDK-supported recipients, 18 other scientists supported by the NIH received the award for their scientific achievements; the NIH has now funded 213 PECASE recipients since the award's inception in 1996. PECASE is the most prestigious award given in the United States to scientists at the outset of their independent research careers.

**Developing Ethical Approaches to Expanding Access to Organ Transplantation**

**Peter P. Reese, M.D., M.S.C.E.**

Dr. Reese, an Assistant Professor of Medicine and Epidemiology at the University of Pennsylvania in Philadelphia, received a 2011 PECASE award in recognition of his contributions to organ transplant research. His primary research focus is in the development of effective strategies to increase access to kidney transplantation. The growing and unmet need for kidney transplants is driven by the rising prevalence of end-stage renal disease (ESRD) in the United States. ESRD impairs quality of life, decreases survival, and is increasingly common among older adults. A shortage of available organs for transplantation has driven strong interest in providing kidney transplants to the patients who benefit the most. Dr. Reese's novel approach will evaluate the use of the patient's functional status—a measure of the ability to complete important daily activities—as a tool to predict which patients derive the greatest survival benefit from a kidney transplant.

**Understanding the Structure and Function of Signaling Cell Surface Receptors**

**Georgios Skiniotis, Ph.D.**

Dr. Skiniotis, a Pew Scholar in the Biomedical Sciences and Research Assistant Professor at the University of Michigan in Ann Arbor, received a 2011 PECASE award for his innovative work using electron microscopy to study the structure of signaling cell surface receptors, such as the leptin hormone receptor and the β2-adrenoceptor. To detect and respond to signaling molecules, cells often employ specialized proteins on their surface termed "receptors." Once a signaling molecule binds, the receptor initiates a cascade of events that results in a cellular response. One of the main areas of research of Dr. Skiniotis's laboratory is on the leptin signaling pathway and its role in the regulation of mammalian energy balance

and body weight. The binding of leptin to the leptin receptor on the cell surface results in the intracellular activation of other signaling proteins, which, in turn, regulate a number of physiological signaling cascades. Dr. Skiniotis and his team have been using electron microscopy techniques to study the molecular architecture of the leptin/leptin receptor complex to understand how the leptin signal is transmitted to the intracellular space. A mechanistic understanding of this complex will inform the design of therapeutic strategies targeting the leptin receptor complex.

*The PECASE awards support the continued professional development of awardees, promote careers and foster innovation in science and technology, and recognize the scientific missions of participating agencies. A list of NIH scientists who have received this prestigious award is available at www.grants.nih.gov/grants/policy/pecase.htm*

# *Healthy Moments*


In addition to supporting biomedical research, the NIDDK engages in education and outreach activities to disseminate science-based health information to patients and their families, health care professionals, and the public. These efforts include targeted outreach to populations disproportionally affected by the diseases and conditions under its purview, including African Americans, Hispanic Americans, and Asian and Pacific Islander Americans. *Healthy Moments* is an example of one such activity.

Featuring NIDDK Director Dr. Griffin Rodgers, *Healthy Moments* is a series of 60-second weekly radio reports that include health information as well as tips on how to prevent and control diseases that are of interest to the Institute. It is broadcast on radio stations that target African American and other minority listeners. The goals of *Healthy Moments* include providing the results and recommendations of NIDDK-supported studies to the public and translating these results for public benefit. Another goal of *Healthy Moments* is to raise awareness locally of patient recruitment efforts for ongoing clinical studies at the NIH. *Healthy Moments* complements the NIDDK's other efforts to disseminate health information to the public, such as the NIDDK Information Clearinghouses and Education Programs, the NIDDK's web-site, and press releases.

Launched in May 2008, *Healthy Moments* began as a weekly radio feature in Washington, DC. Today, more than one and a half million listeners nationwide tune in to these broadcast spots. In addition to the broadcast of weekly episodes, seasonal episodes are produced during back-to-school time, holidays, National Diabetes Month, and National Kidney Month.

*Healthy Moments* features can also be found on the NIDDK web-site at *www2.niddk.nih.gov/HealthEducation/HealthyMoments*. Visitors can listen to past broadcasts and download audio files or transcripts. The *Healthy Moments* web-site can also be accessed by scanning this QR code:

In addition to regular radio spots, *Healthy Moments* is using social media and email to disseminate health information. *Healthy Moments* can be followed on Twitter, @HealthyMoments. The NIDDK Facebook page updates when new *Healthy Moments* episodes are available and key tweets are sent. Through the use of a variety of forms of communication, the NIDDK is working to engage the widest possible audience.

# *NIDDK Training Programs*

**NIDDK Programs Cultivate the Next Generation of Scientists**

Training and developing young researchers is an important goal of the NIH and the NIDDK. NIDDK Director Dr. Griffin Rodgers highlights the fostering of exceptional research training and mentoring opportunities as one of the overarching principles that guide his leadership and vision for the NIDDK. He states, "Maintaining an NIDDK-focused pipeline of outstanding investigators is critically important to our research progress. We will continue to support significant opportunities at the graduate student and postdoctoral levels, as well as through research career development awards, and undergraduate research educational opportunities."

The NIDDK supports research training and career development at a wide range of institutions, both intramural and extramural. NIDDK extramural programs support research training and career development at academic institutions throughout the United States, primarily through funding to the institutions. Through its Intramural Research Program, the NIDDK provides opportunities ranging from summer programs for high school students through employment of postdoctoral researchers on the NIH campus in Bethesda, Maryland, as well as at a Diabetes Epidemiology and Clinical Research Branch in Phoenix, Arizona.

NIDDK-funded research training programs work to maintain a "pipeline" of new investigators at every career stage. Summer training programs provide opportunities for high school and undergraduate students to obtain research experience. The NIH provides support for M.D./Ph.D. and Ph.D. students during the predoctoral phase of their research training and support for research fellows who have received their M.D., Ph.D., or other doctoral-level degree. The NIDDK also supports opportunities for medical students to engage in research. For example, the NIDDK Medical Student Summer Research Program in Diabetes aims to encourage medical students to consider research in diabetes and its complications as a career and to educate students about diabetes.

The NIH has several types of awards to assist career development for physicians engaged in patient-based or basic research and for Ph.D. scientists transitioning to independent positions. Clinical demands make it challenging for physicians to also pursue research careers. Furthermore, there is typically a long process of training and career development before a new independent investigator obtains grant support and leads a research laboratory. Therefore, NIH supports Mentored Patient-oriented Research Career Development Awards, aimed at clinical investigators engaged in patient-based research, and Investigator Awards in Patient-oriented Research to support mid-career physicians who have funded clinical investigations in patient-oriented research and who are mentoring young clinicians. Mentored Research Scientist Development Awards, Mentored Clinical Scientists Development Awards, and the NIH Pathways to Independence awards support scientists transitioning to independent positions. The NIH also provides support to individuals with a quantitative background (*e.g.*, engineering, mathematics, computer science) who wish to pursue biomedical research through the Mentored Quantitative Research Career Development Awards.

Beyond support focused on training and career development, the NIDDK also creates opportunities to help new investigators advance scientific discovery through research project grants for their own laboratories. Each year, the NIDDK sets a percentile "payline" for R01 research project grant applications based on available funds and the volume of applications. The payline is essentially a target threshold for funding based on the percentile score applications receive in the first level of peer review. For "Early Stage Investigators" (a new investigator who has completed his or her terminal research degree or medical residency within the past 10 years), the payline is more generous than the regular payline for established investigators. For example, for fiscal year 2012, the payline for early stage investigator applications was five percentile points higher than the regular payline. In addition, all new investigator R01

applications within 10 percentile points of the payline received individual consideration during the second-level review process by the NIDDK Advisory Council. The NIDDK can also choose to award a 1- or 2-year grant to an R01 application that appears promising but scored outside the payline. These provide support for an investigator to collect preliminary data in order to submit an improved revised R01 application. During second-level review, new investigators are given special consideration for these awards. The NIDDK also regularly holds workshops for recently funded new investigators to provide them with the information they will need to be successful in securing continued support for their research programs.

The NIDDK Office of Minority Health Research Coordination (OMHRC) oversees the NIDDK's efforts to recruit and retain minorities in biomedical research. Several programs provide opportunities for minority students to obtain research experience. For example, through the NIDDK/OMHRC Summer Internship Program for Underrepresented Groups, undergraduate African American, Hispanic and Latino American, American Indian and Alaska Natives, Native Hawaiians, and other Pacific Islanders can participate in a 10 week summer program conducting research at an NIDDK intramural research laboratory. The Alaska Native Undergraduate Summer Internship Program provides research education and training for college students, including Alaska Natives, who have a demonstrated interest in health disparities affecting Alaska Native communities and intend to pursue a career in biomedical research. The program provides 10 weeks of basic or clinical research education and training at the University of Alaska in Anchorage or Fairbanks. In addition, the NIDDK's Short-Term Education Program for Underrepresented Persons (STEP-UP) provides research education grants to seven institutions to coordinate four high school STEP-UP programs and three undergraduate STEP-UP programs that provide students with summer research experience and training opportunities. Further information on opportunities through OMHRC can be found at *www2.niddk.nih.gov/OMHRC/OMHRCResearchTrainingForStudents/OMHRCStudentTraining.htm*

OMHRC has established a communication network of current and potential biomedical research investigators and technical personnel interested in minority health research, including individuals from traditionally under-served communities. The major objective of the Network of Minority Health Research Investigators (NMRI) is to encourage and facilitate participation of members of underrepresented population groups and others interested in minority health in the conduct of biomedical research in the fields of diabetes, endocrinology, and metabolism; digestive diseases and nutrition; and kidney, urologic, and hematologic diseases. A second objective is to encourage and enhance the potential of the investigators in choosing a biomedical research career in these fields. An important component of this network is promotion of two-way communications between network members and the NIDDK. Through the NMRI, the NIDDK elicits recommendations for strategies to enhance the opportunities and implement mechanisms for support of underrepresented population groups and others in biomedical research. The NMRI will advance scientific knowledge and contribute to the reduction and eventual elimination of racial and ethnic health disparities. The annual NMRI Workshop is currently in its 11th year. More information about NMRI is available at: *http://nmri.niddk.nih.gov/*

Research breakthroughs happen only through the efforts of a creative, diverse, and well trained workforce. Thus, the NIDDK will continue programs to train and support researchers at all stages of their careers. The NIDDK will continue to encourage and facilitate participation of members of underrepresented racial and ethnic minority groups in the conduct of biomedical research in NIDDK-relevant fields. This next generation of scientists will then be poised to take advantage of a wealth of research opportunities to improve the health of Americans.

*Additional information on NIDDK-supported opportunities in training and career development can be found on the NIDDK web-site at www2.niddk.nih.gov/Funding/TrainingCareerDev*

## DIVERSE STUDENTS EXPLORE RESEARCH THROUGH NIDDK SUMMER PROGRAMS

On the surface, Camille Miller and Yvonne Johnny appear to have little in common. Camille is an American Indian, lives in the dusty deserts of Arizona, and is enrolled in college to be a registered nurse. Yvonne hails from the tropical paradise of the Federated States of Micronesia (FSM), a U.S.-affiliated territory in the western Pacific Ocean. She hopes to pursue science education when she graduates from high school. But both young women share important similarities: each of their communities suffers from unusually high rates of diabetes and its complications. And each student spent the summer looking for a way to change that.

In the summer of 2012, Camille and Yvonne both participated in NIDDK programs designed to diversify the biomedical research field. Camille studied type 2 diabetes through the NIDDK Summer Internship Program (SIP) at the NIDDK's Phoenix Epidemiology and Clinical Research Branch (PECRB). Yvonne researched type 2 diabetes through the Short-Term Education Program for Underrepresented Persons (STEP-UP) in her native Micronesia. Both presented their research at conferences on the NIH campus in August 2012.

The two programs are designed to provide research opportunities for students from groups underrepresented in biomedical research—including certain racial and ethnic minorities. NIDDK SIP students conduct research related to the NIDDK's mission either at a lab on the main NIH campus or at PECRB, and STEP-UP students work at one of several NIDDK-funded labs in the United States and its territories. In 2012, STEP-UP welcomed students from the Marshall Islands, the FSM, and the U.S. Virgin Islands for the first time, and the NIDDK established a new molecular biology lab in the Marshall Islands.

Dr. Lawrence Agodoa directs the NIDDK's Office of Minority Health Research Coordination, which manages both programs. He said having a diverse pool of researchers to tackle some of science's most pressing issues is crucial.

"People of all walks of life need to come together and think about how to solve these problems," Dr. Agodoa said. "Many chronic diseases such as diabetes affect minority communities disproportionately. Having friends and family who are affected by a disease often gives people extra motivation to pursue biomedical research."

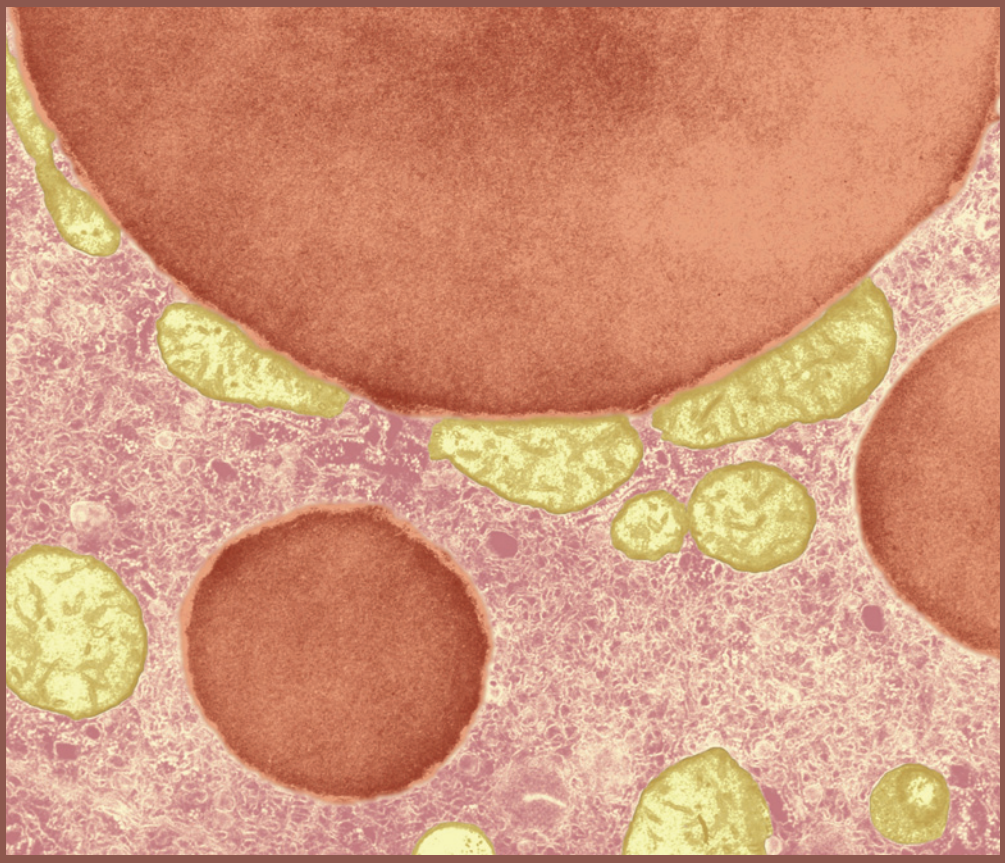
Camille and Yvonne concur. Part of Camille's passion for her research on type 2 diabetes in Pima Indians comes from having experienced the disease within her own American Indian community, the Cocopah Tribe, and even closer to home.

"Diabetes runs in my family, and my grandparents were amputees from diabetes complications. They ended up dying from diabetes. I really think that my family and tribal members could benefit from my research," she said.

The research that Camille conducted this summer at PECRB was her first foray into the world of biomedical research, and she hopes to apply for an NIH grant one day to resume her investigations.

Yvonne also hopes to continue her research.

"I want to find a cure for diabetes to help my family and friends back home," she said. "I see myself as the future of diabetes research."



**Mitochondria are small structures inside of cells that generate much of the “fuel” cells use to perform their functions. In this image, several mitochondria (yellow) attach to a fat droplet (red) in a liver cell. This attachment may facilitate transport of fat molecules into the mitochondria where they are metabolized into energy for the cell.**

*The Cell 2nd edition. Credit: Don W. Fawcett / Science Source*

# Diabetes, Endocrinology, and Metabolic Diseases

*NIDDK support of basic and clinical research in the areas of diabetes, endocrinology, and metabolic diseases spans a vast and diverse range of diseases and conditions, including diabetes, osteoporosis, cystic fibrosis, and obesity. Together, these diseases and conditions affect many millions of Americans and can profoundly decrease quality of life. Many of these diseases are complex—an interplay between genetic and environmental factors contributes to disease development.*

Diabetes is a debilitating disease that affects an estimated 25.8 million people in the United States—or 8.3 percent of the total population—and is the seventh leading cause of death.[1] Diabetes lowers average life expectancy by up to 15 years,[2] increases risk of death from cardiovascular disease 2- to 4-fold, and is the leading cause of kidney failure, nontraumatic lower limb amputations, and, in working-age adults, blindness.[1] In addition to these human costs, the estimated total financial cost for diabetes in the United States in 2007—including costs of medical care, disability, and premature death—was $174 billion.[1] Effective therapy can prevent or delay diabetic complications, but approximately one-quarter of Americans with diabetes are undiagnosed and therefore not receiving therapy.[1]

Diabetes is characterized by the body's inability to produce and/or respond appropriately to insulin, a hormone that is necessary for the body to absorb and use glucose (sugar) as a cellular fuel. These defects result in persistent elevation of blood glucose levels and other metabolic abnormalities, which in turn lead to the development of disease complications. The most common forms of diabetes are type 1 diabetes, in which the body loses its ability to produce insulin; and type 2 diabetes, in which the body becomes resistant to insulin signaling, with subsequent impaired insulin production. In addition, a significant proportion of pregnant women each year are diagnosed with gestational diabetes, a form of diabetes that is similar to type 2 diabetes but unique to pregnancy. Untreated, any form of diabetes during pregnancy increases the risk of serious complications for the mother and baby before, during, and after delivery.

Type 1 diabetes, formerly known as juvenile diabetes, affects approximately 5 percent of adults and the majority of children and youth with diagnosed diabetes.[1] It most often develops during childhood, but may appear at any age. Type 1 diabetes is an autoimmune disease in which the immune system launches a misguided attack and destroys the insulin-producing beta (β) cells of the pancreas. If left untreated, type 1 diabetes results in death from starvation: without insulin, glucose is not transported from the bloodstream into the body's cells, where it is needed. Thus, patients require lifelong insulin administration—in the form of multiple daily injections or via an insulin pump—in order to regulate their blood glucose levels. Despite vigilance in disease management, with frequent finger sticks to test blood glucose levels and the administration of insulin, it is still impossible for patients to control blood glucose levels to near the normal levels achieved by functional β cells. Thus, researchers are actively seeking new

[1] *2011 National Diabetes Fact Sheet. Centers for Disease Control and Prevention. Atlanta, GA.*

[2] *Portuese E and Orchard T: Mortality in Insulin-Dependent Diabetes. In Diabetes in America (pp. 221-232). Bethesda, MD: National Diabetes Data Group, NIH, 1995.*

methods to improve blood glucose monitoring and insulin delivery, as well as working to develop β cell replacement therapies to cure type 1 diabetes.

Type 2 diabetes is the most common form of the disease, accounting for about 90 to 95 percent of diagnosed diabetes cases in U.S. adults.[1] Type 2 diabetes is associated with several factors, including older age and a family history of the disease. It is also strongly associated with obesity; more than 80 percent of adults with diabetes are overweight or obese.[3] Type 2 diabetes occurs at elevated rates among minority groups, including African Americans, Hispanic and Latino Americans, American Indians, and Native Hawaiians and Pacific Islanders.[1] Gestational diabetes is also a risk factor: women who have had gestational diabetes have a 35 to 60 percent chance of developing diabetes—mostly type 2 diabetes—in the next 10 to 20 years.[1]

In people with type 2 diabetes, cells in muscle, fat, and liver tissue do not properly respond to insulin. As a result, the pancreas initially produces more insulin to compensate. Gradually, however, the pancreatic β cells lose their ability to secrete enough insulin to restore balance, and the timing of insulin secretion becomes abnormal, causing blood glucose levels to rise. Treatment approaches for controlling glucose levels include diet, exercise, and oral and injected medications, with insulin often required as the disease progresses. There are also an estimated 79 million adults in the United States who have a condition called "prediabetes," in which blood glucose levels are higher than normal, but not as high as in diabetes.[1] This population is at high risk of developing diabetes. Fortunately, the NIDDK-supported Diabetes Prevention Program (DPP) clinical trial has shown that people with prediabetes can dramatically reduce their risk of developing type 2 diabetes with diet and exercise changes designed to achieve a 7 percent reduction in body weight. Moreover, follow-up research has shown that this benefit of reduced diabetes risk can persist for at least 10 years.

Type 2 diabetes was previously called "adult-onset" diabetes because it is predominantly diagnosed in older individuals. However, this form of diabetes is increasingly being diagnosed in children and adolescents, and it disproportionately affects minority youth. Believed to be related to increasing rates of pediatric obesity, this is an alarming trend for many reasons. For example, the NIDDK-supported Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) clinical trial showed that the disease may be more aggressive and difficult to treat in youth compared to adults. This is worrisome because the onset and severity of disease complications correlate with both the duration of diabetes and control of blood glucose levels; thus, those with early disease onset are at greater risk with respect to complications than those who develop the disease later in life. Because diabetes often becomes more difficult to control over time, people diagnosed in their youth may find it even more challenging to control their blood glucose levels as they get older and thus prevent or delay the development of complications. In addition, increasing rates of type 2 diabetes in girls may lead to increasing numbers of women who enter pregnancy with diabetes, and maternal diabetes during pregnancy—either onset of type 2 diabetes before pregnancy or the development of gestational diabetes during pregnancy—confers an increased risk of type 2 diabetes in offspring. Thus, the rising rates of diabetes and prediabetes in young women could lead to a vicious cycle of ever-growing rates of diabetes. Therefore, the advent of type 2 diabetes in youth has the potential to worsen the enormous health burden that diabetes already places on the United States.

The NIDDK is supporting research to better understand metabolism and the mechanisms that lead to the development and progression of diabetes and the many other endocrine and metabolic diseases within the NIDDK's mission; such research will ultimately spur the design of potential new intervention strategies. Exploring interrelationships between some of these diseases is an important and informative facet of this work—for example, diabetes is becoming an increasing problem for people with cystic fibrosis, as life expectancy for these individuals has improved due to advances in cystic fibrosis treatment. In parallel, based

[3] *Eberhardt MS, et al. MMWR 53: 1066-1068, 2004.*

on knowledge from past scientific research investments, the NIDDK is vigorously pursuing studies of prevention and treatment approaches for these diseases.

## GENETICS OF TYPE 2 DIABETES

**Uncovering Genetics Contributing to Diabetes Health Disparities:** Researchers scanning the genome have uncovered genetic factors that may account for at least part of the elevated risk for type 2 diabetes in African American and South Asian populations. Detailed maps of common genetic variation derived from the Human Genome and HapMap projects have led to a renaissance in the ability to study the genetics of human disease. For example, geneticists using the genome-wide association studies (GWAS) approach have found over 60 gene regions that affect risk for type 2 diabetes. However, while genetic factors are thought to contribute significantly to the higher burden of the disease in many non-white populations, the early studies focused on people of European descent. This was because the genome maps originally designed for GWAS analysis had not adequately captured genetic variation common in non-white populations to permit these studies. To better understand how genes affect diabetes health disparities, researchers have redesigned these tools and begun using them to perform GWAS with samples that are specific to populations with the highest burden of type 2 diabetes.

In one study, researchers used GWAS to look for genetic differences between African Americans who either had or did not have type 2 diabetes. Genetic markers that were more common in one group than in the other were then confirmed using samples from a larger number of African American study participants. This approach led to the discovery of one novel genomic location that appears clearly to influence the risk for type 2 diabetes in African Americans, as well as four other genomic locations that may be involved, although the data are less certain.

In a separate study, researchers examined DNA samples from South Asians, a population which also has elevated risk for type 2 diabetes relative to people of European heritage. This approach led to the discovery of six genomic regions affecting type 2 diabetes risk in South Asian peoples. One of these regions includes *GRB14*, a gene encoding a protein that binds the insulin receptor and is thought to affect insulin sensitivity. Mice lacking *GRB14* are lean, and more sensitive to insulin than normal mice. Another region includes *HNF4A*, a gene in which previously discovered mutations were found to reduce pancreatic insulin production and cause a rare disease called maturity onset diabetes of the young. While neither study reported discovering the precise genetic differences within the identified genomic regions that promote type 2 diabetes in African Americans or South Asians, or that provide Caucasians with relative protection from the disease, further studies looking in the 11 genomic regions identified by the 2 studies could begin to explain the cause of type 2 diabetes health disparities.

*Palmer ND, McDonough CW, Hicks PJ, et al. A genome-wide association search for type 2 diabetes genes in African Americans. PLoS One 7: e29202, 2012.*

*Kooner JS, Saleheen D, Sim X, et al. Genome-wide association study in individuals of South Asian ancestry identifies six new type 2 diabetes susceptibility loci. Nat Genet 43: 984-989, 2011.*

**Understanding the Genetic Risk for Type 2 Diabetes:** Analyses of genetic variations associated with type 2 diabetes provide insight into how these variations affect an individual's risk for this disease. An explosion of technologies and tools has enabled researchers to conduct large studies to compare the genomes of thousands of people with and without type 2 diabetes to identify genetic variants that affect the likelihood of developing the disease. Whereas genetic risk for some diseases can be accounted for by a few variants with large effects, the current set of over 60 risk variants for type 2 diabetes only explains a small percentage of the genetic risk for the disease. In addition, how most of these variants alter the risk of type 2 diabetes is unknown. Toward the goal of elucidating the effects of these variants on disease risk, researchers are utilizing a number of

different approaches, taking advantage of the latest developments in tools and technologies, as evidenced by two recent studies.

In the first study, scientists hypothesized that identification of additional risk variants and the function of the genes they affect will illuminate a limited set of molecular pathways that influence development of type 2 diabetes. By conducting a meta-analysis in which they pooled data from over 34,000 people with type 2 diabetes and nearly 115,000 people without the disease, the researchers identified 10 previously unreported type 2 diabetes risk variants. Two of the newly discovered associated variants showed sex-differentiated associations; one was more significantly associated with type 2 diabetes in males, and the other in females. The researchers used several analytical approaches to identify pathways and networks that may be involved in the development of type 2 diabetes. The investigators found that some of the key processes influenced by type 2 diabetes risk genes include regulation of cell division, signaling by proteins secreted by fat cells, and regulation of gene activity (whether a gene is turned "on" or "off") by a protein called CREBBP.

In another study, the researchers focused on assessing the extent to which the previously identified type 2 diabetes genetic risk variants affected the activity of genes that are either nearby or distant from them (often on a different chromosome). Genes are typically regulated in the sense that they can be kept inactive, when not needed, or utilized to varying degrees. The regulatory elements—which typically include DNA near the gene and the proteins that interact with that DNA—are thus analogous to the components of a dimmer switch: they control the extent to which a gene is activated (or "expressed") in any given cell. The investigators sought to determine which of the known type 2 diabetes risk variants affect expression levels of nearby genes, which affect expression of distant genes, and which have little impact on gene expression. They found that, in general, genetic variants associated with type 2 diabetes affected genes at a distance from the genetic variation itself—either on a different chromosome than that containing the variant or on the same chromosome, but not nearby. This result is surprising because many researchers assumed that the identified genetic variants altered the activity of a gene or genes in the same chromosomal neighborhood as the variant. Because specific genes are often more active in one tissue than in another, the investigators examined the impact of the type 2 diabetes risk genes on gene expression in multiple tissues involved in the disease. The data revealed many tissue-specific effects, which may eventually help uncover how specific genetic variants exert their effects on disease risk. By increasing understanding of the genetic factors that influence development of type 2 diabetes, researchers hope to use this knowledge to tailor prevention and treatment strategies to individuals and to develop new therapeutic approaches.

*Morris AP, Voight BF, Teslovich TM, et al. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nat Genet 44: 981-990, 2012.*

*Elbein SC, Gamazon ER, Das SK, Rasouli N, Kern PA, and Cox NJ. Genetic risk factors for type 2 diabetes: a trans-regulatory genetic architecture? Am J Hum Genet 91: 466-477, 2012.*

## EXAMINING COST-EFFECTIVENESS OF APPROACHES TO PREVENT TYPE 2 DIABETES

**Interventions To Prevent Type 2 Diabetes Provide Good Return on Investment:** New research has shown that using either of two interventions to prevent or delay type 2 diabetes in people at high risk for the disease would be a very cost effective way to improve their health and quality of life. The landmark Diabetes Prevention Program (DPP) clinical study demonstrated that an intensive lifestyle intervention designed to achieve modest weight loss through a combination of diet and exercise lowered type 2 diabetes rates by 58 percent, and that the generic diabetes medication metformin reduced diabetes rates by 31 percent, relative to placebo. Subsequently, researchers with the follow-up DPP Outcomes Study (DPPOS) showed that the health benefits of both DPP interventions persisted

for at least 10 years. In the new report, DPP researchers examined per person costs of the interventions during the trial and follow up, total direct medical costs outside the DPP/DPPOS, and measures of quality of life over 10 years. They found that the lifestyle intervention was cost-effective—that is, its modest net cost was well justified by the benefits of diabetes prevention, overall improvements in health, and the reduction in other health care costs. The new research also found that, although health benefits from metformin treatment were more limited than those conferred by the lifestyle intervention, the use of this inexpensive drug in the DPP population yielded a modest cost savings. The greater reduction in health care costs from the more expensive lifestyle intervention was nearly enough to offset the cost of the intervention, so that the lifestyle intervention was highly cost effective and nearly cost neutral. Previous research showed that metformin was most effective among DPP participants who were less than 60 years of age when the trial began, and among those with a history of gestational diabetes.

Throughout the study, quality of life as measured by mobility, level of pain, emotional outlook, and other indicators was consistently best in the lifestyle group, compared to metformin or placebo. These findings are particularly encouraging in light of other NIDDK-supported research demonstrating the feasibility of substantially reducing the cost of the lifestyle intervention by delivering it to groups in community-based settings such as YMCAs. It is hoped that this approach will yield both health care savings and better health for many people, if widely implemented.

*Diabetes Prevention Program Research Group. The 10-year cost-effectiveness of lifestyle intervention or metformin for diabetes prevention: an intent-to-treat analysis of the DPP/DPPOS. Diabetes Care 35: 723-730, 2012.*

## TREATING TYPE 2 DIABETES IN YOUTH

**Trial Highlights Challenge in Treating Youth with Type 2 Diabetes:** Results reveal that type 2 diabetes is more difficult to treat in youth with the disease than in adults. Although type 2 diabetes is most commonly diagnosed in people over the age of 40, an increase in childhood obesity and other factors has led to a significant rise in cases in people under 20 years of age. Since development of long-term complications in adults is related to both duration of diabetes and control of blood glucose levels, the increasing prevalence of people diagnosed with type 2 diabetes during childhood is a major public health concern. Prior to this study, it was unknown whether treatments developed for adults would work well for younger patients. Metformin, the first-line drug of choice among adults with type 2 diabetes, is currently the only oral medication approved for use in children.

The Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) trial tested how well three treatment approaches controlled blood glucose levels in youth ages 10 to 17. At the start of the study, the 699 participants had type 2 diabetes for less than 2 years and were either overweight or obese (as measured by a body mass index at the 85$^{th}$ percentile or greater). Participants were randomly assigned to receive either metformin alone; metformin and another diabetes drug, rosiglitazone, together; or metformin plus intensive lifestyle changes aimed at helping participants lose weight and increase physical activity. Unfortunately, metformin alone failed to maintain acceptable, long-term blood glucose control in 51.7 percent of youth over an average follow-up of 46 months—a much higher failure rate than expected. Metformin plus lifestyle changes failed 46.6 percent of the time, an improvement over metformin alone that is not statistically significant. The combination of metformin plus rosiglitazone was significantly better, but still failed 38.6 percent of the time over the follow-up period. Importantly, after the study began, the U.S. Food and Drug Administration restricted use of rosiglitazone because of studies linking the medicine to a higher risk of heart attacks and stroke in adults. Thus, at this point, rosiglitazone is not recommended for use in children.

These results suggest treatment with metformin alone may be inadequate for a majority of youth with type 2 diabetes, and that type 2 diabetes is a more aggressive disease in youth than in adults. The findings also emphasize the importance of preventing and treating

childhood obesity, so that overweight and obese youth do not develop type 2 diabetes. Most of the medications widely used by adults to control type 2 diabetes have not been studied in children. Additional research is needed to determine whether any of these, singly or in combination, can safely and reliably control blood glucose in young people with the disease.

*Zeitler P, Hirst K, Pyle L, et al. A clinical trial to maintain glycemic control in youth with type 2 diabetes. N Engl J Med 366: 2247-2256, 2012.*

## MONITORING CHILDREN AT RISK FOR TYPE 1 DIABETES

**Research on Preventing a Life-threatening Condition Associated with Type 1 Diabetes Onset:** Researchers have shown that young children being monitored from birth in a long-term research study have a reduced risk of developing diabetic ketoacidosis (DKA) at onset of type 1 diabetes compared to children in the general population. DKA is caused by profound insulin deficiency. When symptoms of diabetes are not recognized, the disease can progress to life-threatening DKA. Scientists estimate that as many as 40 percent of children who develop type 1 diabetes under age 3 and 60 percent of children under age 2 have DKA at disease onset. This condition could lead to coma or death, underscoring the need to find ways to prevent it. Toward that goal, researchers examined whether children participating in The Environmental Determinants of Diabetes in the Young (TEDDY) study had reduced rates of DKA. TEDDY is following over 8,000 newborns at high genetic risk of developing type 1 diabetes until they are 15 years old to identify environmental triggers of disease. Researchers hypothesized that children in TEDDY may have lower rates of DKA at disease onset because they are being monitored frequently and the parents know that their child is at genetic risk of developing type 1 diabetes, both of which could lead to earlier diagnosis and prevention of DKA.

To determine whether participants in TEDDY have lower rates of DKA, researchers compared children who were diagnosed with the disease in TEDDY with children who were diagnosed in the general population of countries where TEDDY is being conducted (United States, Germany, Finland, Sweden), using data from studies and national registries. In children diagnosed under age two, the percent with DKA was significantly lower in TEDDY than in the general population. In children diagnosed under age five, the percent with DKA was lower in TEDDY compared to the general populations in the United States and Germany, but not different in Finland and Sweden, where the disease is more common. The research suggests that DKA occurs less frequently in TEDDY children. The scientists note that it is currently cost-prohibitive to do the type of screening and monitoring being done in TEDDY on a population-wide scale. However, the research sheds light on approaches that could be used in the future to achieve the goal of preventing this life-threatening condition.

*Elding Larsson H, Vehik K, Bell R, et al. Reduced prevalence of diabetic ketoacidosis at diagnosis of type 1 diabetes in young children participating in longitudinal follow-up. Diabetes Care 34: 2347-2352, 2011.*

## CONTROLLING BLOOD GLUCOSE CAN PREVENT LOSS OF KIDNEY FUNCTION

**Intensive Blood Glucose Control Reduces Kidney Disease:** New results show that controlling blood glucose early in the course of type 1 diabetes yields huge dividends, preserving kidney function for decades. The landmark Diabetes Control and Complications Trial (DCCT) began in 1983, but because it can take years for early signs of diabetes complications to develop, it was not until 1993 that sufficient time had passed for the trial to prove that intensive blood glucose control reduced early signs of kidney dysfunction and other complications in people with type 1 diabetes. However, because more serious impairment of kidney function or kidney disease can take even longer to develop, researchers could not determine the effect of intensive therapy on the development of kidney disease at that time. DCCT participants were invited to join the DCCT follow-up, the Epidemiology of Diabetes Interventions and Complications (EDIC) study, and

today, nearly 3 decades after the start of the DCCT, about 95 percent of DCCT participants continue to be followed to determine the long-term effects of the therapies beyond the initial treatment period. Now, after an average 22-year follow-up, EDIC researchers reported that controlling blood glucose can prevent loss of kidney function and is likely to reduce kidney failure. Compared to conventional therapy, near-normal control of blood glucose—beginning soon after diagnosis of type 1 diabetes and continuing an average 6.5 years—reduced the long-term risk of developing kidney disease by 50 percent. This finding—along with previous DCCT/EDIC research demonstrating the benefit of intensive blood glucose control in reducing the risk of eye, nerve, and cardiovascular complications—reinforces the importance of early, intensive blood glucose control in people with type 1 diabetes. DCCT and EDIC illustrate the value of long-term studies, have revolutionized disease management, and led to greatly improved outcomes for people with type 1 diabetes.

*de Boer IH, Sun W, Cleary PA, et. al. Intensive diabetes therapy and glomerular filtration rate in type 1 diabetes. N Engl J Med 365: 2366-2376, 2011.*

## NEW APPROACHES TO PREVENT AUTOIMMUNITY

**Small Molecules Hold Promise To Prevent Type 1 Diabetes:** New research has identified promising small molecules to prevent development of type 1 diabetes. Because type 1 diabetes results from inappropriate activity of the immune system, scientists are pursuing potential treatments that suppress this harmful activity. However, many agents that suppress the harmful aspects also suppress the protective aspects of the immune system and, therefore, can have toxic side effects and increase a person's risk for infection. Scientists are investigating therapies to selectively suppress the specific cells involved in autoimmune diseases like type 1 diabetes. Small molecules have proven valuable for affecting the function of genes, cells, and biological pathways, and hold great promise for the prevention of type 1 diabetes. A key challenge, however, is to identify small molecules that can selectively modulate a specific biological process or disease state. Two recent reports utilized different strategies to find promising new molecules for the prevention of type 1 diabetes.

In previous work, a group of scientists developed a small molecule that selectively affected Th17 cells—immune system cells that produce a protein called IL-17 and have been previously implicated in autoimmune diseases. They demonstrated that the molecule affected Th17 cells by binding to and repressing two proteins, called RORα and RORγ, whose activity is required for the development of Th17 cells. In new research, these scientists synthesized a modified version of their molecule that selectively binds to and suppresses RORγ, but not RORα. This was done because other research suggested that suppressing RORγ alone inhibits development of Th17 cells, and it is preferable to develop small molecules that specifically target the disease process, to reduce the chance of adverse side effects. This new molecule still inhibits IL-17 production, suggesting that the molecule is a potent repressor of RORγ activity and has potential for the treatment of autoimmune disease.

Another group of scientists, searching for a potential drug to prevent type 1 diabetes, screened a large library of small molecules and identified one that alters a key autoimmune reaction in this disease. With knowledge of the structure of a protein that confers type 1 diabetes risk (a form of the MHC class II protein), the researchers used computer simulation to identify small molecules that are likely to bind this protein, and then tested these molecules further in experiments with cells and mice. They hypothesized that these small molecules, by binding the protein, could alter how the protein presents insulin to immune cells called T cells, which is important in the development of autoimmunity. Indeed, further analyses demonstrated that certain of these small molecules could enhance or inhibit the T cell response to insulin; enhancing certain types of T cell responses could potentially prevent or dampen autoimmunity. One small molecule they identified, called glyphosine, enhanced a T cell response to insulin in mouse cells, increased production of an anti-inflammatory protein (called IL-10),

and, when given to mice genetically susceptible to type 1 diabetes, prevented development of the disease. Importantly, giving glyphosine to human cells from people with type 1 diabetes suggested that it may enhance a protective type of T cell response in humans as well. Further research with these promising small molecules will determine whether they have utility in humans to prevent type 1 diabetes.

*Kumar N, Lyda B, Chang MR, et al. Identification of SR2211: a potent synthetic RORγ-selective modulator. ACS Chem Biol 7: 672-677, 2012.*

*Michels AW, Ostrov DA, Zhang L, et al. Structure-based selection of small molecules to alter allele-specific MHC class II antigen presentation. J Immunol 187: 5921-5930, 2011.*

**Homing in on Mechanisms To Promote Immune Tolerance—Implications for Combating Autoimmune Diseases:** Researchers discovered new details of a molecular mechanism that is critical to promoting immune tolerance of the body's own tissues, organs, and cells. In autoimmune diseases, like type 1 diabetes, the immune system launches a misguided attack against substances and tissues normally found in the body. In a person without autoimmune disease, immune cells are normally generated that recognize the body ("self") in addition to those that recognize foreign entities such as pathogenic bacteria or virus. In a process called "tolerance," these self-recognizing cells are muted or destroyed. Loss of tolerance, therefore, plays a role in the development of autoimmune diseases, and understanding how tolerance works and what goes wrong is critical to prevent and treat autoimmune diseases.

Previous research shed light on this process with the discovery that a protein, called AIRE, is critical in promoting tolerance. AIRE, by turning genes on and off, generates a wide variety of "self" proteins in the thymus. The presence of these "self" proteins in the thymus promotes tolerance. However, AIRE does not generate all the "self" proteins that are present in the body, so researchers hypothesized that there must be AIRE-independent mechanisms at work. To examine other potential mechanisms, scientists focused on understanding the role that a specific type of immune cell—a dendritic cell—plays in promoting tolerance. Dendritic cells can be found in immune organs like the thymus, and at interfaces between the body and the environment, like skin or the surfaces of the airway and intestine. These cells constantly "sample" the environment; they take up proteins and particulates and then "present" the sample to cells that determine whether or not to launch an immune attack.

The researchers studied one type of dendritic cell, a plasmacytoid dendritic cell (pDC). They examined this cell type because they previously found that it contained a protein called CCR9, which is involved in homing of other immune cells to the thymus. Therefore, they speculated that CCR9 may also be involved in homing pDCs to the thymus. To test this hypothesis, they genetically engineered mice to lack the CCR9 gene and found that these mice had fewer thymic pDCs compared to normal mice, suggesting that CCR9 was important for pDC recruitment to the thymus. In addition, they intravenously injected mice with pDCs without CCR9 and observed that only a small percentage made it to the thymus. The researchers next wanted to determine whether pDCs had a role in transporting "self" proteins to the thymus. They loaded pDCs with an experimental "self" protein and injected those cells into genetically modified mice. The injected pDCs homed to the thymus and, importantly, led to the destruction of immune cells that recognized the "self" protein. Further experiments showed that pDCs within mice were able to "pick up" an experimentally introduced particulate and transport it to the thymus, a possible therapeutic approach for inducing tolerance.

These results suggest that pDCs contribute to tolerance through CCR9-dependent transport of "self" proteins to the thymus, a process that complements AIRE-regulated mechanisms. Further research will determine whether a similar mechanism occurs in humans and whether disruption of this process is associated with type 1 diabetes and/or other autoimmune diseases. This finding could also present an exciting opportunity to identify novel approaches to promote tolerance in autoimmune diseases.

*Hadeiba H, Lahl K, Edalati A, et al. Plasmacytoid dendritic cells transport peripheral antigens to the thymus to promote central tolerance. Immunity 36: 438-450, 2012.*

## BETA CELLS AND DIABETES

**Beta Cell Markers of Maturity:** Researchers have discovered new markers that allow them to distinguish between mature β cells that produce insulin in a regulated fashion and immature β cells that do not. A major goal of diabetes research is to turn stem and/or progenitor cells in the laboratory into insulin-producing β cells that can be transplanted into people. Importantly, those cells must release insulin properly in response to glucose to be a viable therapeutic option. Significant progress has been made in generating immature β cells in the lab, but these cells do not respond appropriately to glucose. In order to develop strategies to turn immature β cells into mature cells, it would be useful if scientists had experimental markers that helped them distinguish between the two cell types. The authors discovered that immature cells isolated from newborn mice released insulin in response to lower glucose levels than mature cells isolated from adult mice. In other words, the immature cells had a lower "glucose threshold" for releasing insulin. The researchers then compared how the levels of various proteins differed between the two groups of cells. They found that a protein called urocortin 3 was present at much higher levels in functionally mature β cells than in immature cells in both mice and humans. These discoveries provide important new tools to scientists testing experimental strategies to generate mature β cells in the lab—they could use differences in glucose threshold and urocortin 3 levels as markers of β cell maturation. These findings can facilitate research to turn stem and/or progenitor cells into mature β cells as a possible therapy for people with diabetes.

*Blum B, Hrvatin SS, Schuetz C, Bonal C, Rezania A, and Melton DA. Functional beta-cell maturation is marked by an increased glucose threshold and by expression of urocortin 3. Nat Biotechnol 30: 261-264, 2012.*

**Signaling Pathway Found To Regulate Regeneration of Insulin-producing β Cells:** Two recent studies identified a signaling pathway that regulates β cell regeneration and could be targeted for new therapies. β cells, which produce insulin, are destroyed by the immune system in people with type 1 diabetes and may not function normally in people with type 2 diabetes. Identifying ways to replace the β cells and restore insulin-producing capacity could benefit people with type 1 or type 2 diabetes, and is a major goal of research. One approach to replace β cells is through regeneration, such as by coaxing existing β cells, which reside in the pancreas, to proliferate and generate new β cells. In particular, small molecules that could promote β cell regeneration could be therapeutically useful, particularly if they had limited off-target effects and could be taken orally. Toward the goal of identifying such small molecules, two different research groups participating in the Beta Cell Biology Consortium conducted screens to identify small molecules that enhanced β cell regeneration.

In one study, researchers screened over 7,000 small molecule compounds in a zebrafish model in which β cells were partially destroyed through genetic manipulation. They identified five compounds that doubled the number of β cells in the animals. Interestingly, four of the five compounds targeted the same cellular pathway—the adenosine signaling pathway. Further experiments focused on the most potent compound, called NECA. NECA was found to promote β cell proliferation specifically, without significantly affecting proliferation of other pancreatic cell types (*e.g.*, glucagon-producing cells) or cells in other tissues (*e.g.*, liver, gut). To determine whether NECA had a similar effect in mammals, the researchers studied an adult mouse model of diabetes in which β cells were depleted. After 15 days, β cell mass was 8-fold larger in the NECA-treated mice compared to control mice, suggesting that NECA promotes β cell regeneration in the mouse model as well.
In a separate study, researchers developed a high-throughput small molecule screening system using rat islets, and used that experimental platform to screen about 850 compounds for their ability to

increase β cell proliferation. Two compounds increased β cell proliferation 2- to 3-fold above control levels; the compounds also increased β cell proliferation in mouse and pig islets. Both of the compounds are inhibitors of adenosine kinase, an enzyme in the adenosine signaling pathway. They tested one of the compounds in mice, and it increased β cell proliferation but did not affect proliferation of other cell types tested, suggesting that inhibiting adenosine kinase may selectively promote β cell proliferation.

Both studies point to the adenosine signaling pathway as a key regulator of β cell regeneration. The preliminary results with the compounds tested in these studies are promising because they may promote β cell proliferation selectively, which would be critically important in any therapy used in people. The research suggests that therapies targeting this pathway could potentially be used to promote β cell regeneration to treat diabetes.

*Andersson O, Adams BA, Yoo D, et al. Adenosine signaling promotes regeneration of pancreatic β cells in vivo. Cell Metab 15: 885-894, 2012.*

*Annes JP, Ryu JH, Lam K, et al. Adenosine kinase inhibition selectively promotes rodent and porcine islet β-cell replication. Proc Natl Acad Sci USA 109: 3915-3920, 2012.*

**Small Molecule Enhances β Cell Function:** Researchers have discovered a family of small molecules that increases insulin production in pancreatic β cells, and thus could be explored for potential use in diabetes therapy. They built on previous research showing that small molecules called isoxazoles (Isx) increase levels of the NeuroD1 protein in nerve cells and promote neuronal cell development. NeuroD1 is also a key regulator of β cell development and maturation, as well as insulin production in β cells. Therefore, the scientists tested whether Isx had an effect on β cells, which are found in clusters called islets in the pancreas. They examined human islets that had been in laboratory culture for a long period of time (2-12 months), during which they lose their ability to secrete insulin in response to glucose. They discovered that treating these human islets with Isx increased the levels of cellular factors that regulate insulin production, β cell function, and β cell development, resulting in enhanced insulin production. Experiments using mouse pancreatic cells in culture showed that treatment with Isx stimulated insulin secretion in response to glucose. These findings make Isx one of only a few known molecules that improves β cell function dramatically, although further research is needed before Isx or related molecules could be tested in people. Because impaired β cell function is central to both type 1 and type 2 diabetes, this research could potentially lead to new therapeutic approaches for both forms of the disease.

*Dioum EM, Osborne JK, Goetsch S, Russell J, Schneider JW, and Cobb MH. A small molecule differentiation inducer increases insulin production by pancreatic β cells. Proc Natl Acad Sci USA 108: 20713-20718, 2011.*

## RESEARCH ON DIABETIC NERVE PAIN

**Newly Discovered Pathway for Nerve Pain in Diabetes:** A factor produced during glucose metabolism may be responsible for painful peripheral neuropathy in diabetes. Many people with diabetes suffer from peripheral neuropathy, nerve damage that starts in the feet and can cause either pain or loss of feeling in the toes and then feet, legs, and hands. While scientists have suspected that elevated blood glucose levels play a role in painful peripheral neuropathy, the exact mechanism(s) has been unclear. In a recent study, researchers found that, compared to people without diabetes, people with type 2 diabetes have higher circulating levels of methylglyoxal, a small molecule that is produced during glucose metabolism. Intriguingly, people with diabetes and foot pain had significantly higher levels of this molecule than either people without diabetes or people with diabetes but no pain. Methylglyoxal is metabolized by a cellular enzyme called glyoxylase 1, or GLO1. Peripheral nerves, such as those detecting sensations in the hands and feet, have low GLO1 activity; because there may be insufficient GLO1 to metabolize excess methylglyoxal, these nerves may be particularly vulnerable to accumulating high levels

of the molecule. To determine whether methylglyoxal exacerbates pain responses, the researchers used a variety of approaches to experimentally raise the levels of this molecule in mice, including chemically inducing diabetes in some and inhibiting the GLO1 enzyme activity in others. They found that, compared to untreated animals, these mice became hypersensitive to both heat and mechanical stimuli. In contrast, the scientists were able to "rescue" diabetic mice from hypersensitivity to heat either by increasing GLO1 enzyme levels in peripheral nerves, or by injecting the mice with a molecule that gets rid of excess amounts of methylglyoxal. Through additional experiments, the researchers uncovered evidence for a molecular mechanism that could explain how exposure to excess methylglyoxal increases the excitability of pain-signaling peripheral nerve cells and enhances activation of brain regions involved in pain processing.

At this time, few effective treatments exist for painful diabetic neuropathy. The novel discovery of a metabolically driven mechanism that increases sensitivity to potentially painful stimuli not only opens up a new line of study, but could potentially lead to new therapeutic approaches for this pain condition in people with diabetes.

*Bierhaus A, Fleming T, Stoyanov S, et al. Methylglyoxal modification of Nav1.8 facilitates nociceptive neuron firing and causes hyperalgesia in diabetic neuropathy. Nat Med 18: 926-933, 2012.*

## METABOLIC REGULATORS OF HEALTH AND DISEASE

**Switching Fuel Sources from Fat to Sugar—Novel Role for Mitochondrial Enzyme and Implications for Type 2 Diabetes:** Researchers have discovered an important role for an enzyme called carnitine acetyltransferase (CrAT) in regulating fuel selection under different nutritional conditions. CrAT is located in mitochondria, the structures in cells that extract energy from a variety of fuel sources. Glucose is their primary fuel, but mitochondria can switch to burning fat when glucose levels are low, such as during a fast. Previous research showed that the mitochondria of people with type 2 diabetes are not as adept as those of healthy people when it comes time to switch back to using glucose following a meal—and also suggested that the reason may relate to a problem with CrAT. The major function of CrAT is to catalyze the transfer of the "acetyl" portion of a molecule called acetyl-CoA to an essential nutrient called carnitine. Unlike CoA, carnitine can take acetyl groups out of the mitochondria. This is important, because acetyl-CoA is a major by-product of mitochondrial energy production, and, if it accumulates, it can interfere with the work of other mitochondrial enzymes, including those involved in glucose metabolism. The new research explores the role of CrAT in metabolism, and helps explain why fuel switching is reduced in people with type 2 diabetes.

The researchers genetically engineered mice to lack CrAT in their muscles, finding that such animals became insulin resistant and had high blood glucose levels, two conditions associated with prediabetes and diabetes. These results suggested that CrAT was playing a role in regulating metabolism. Experiments on mitochondria isolated from the animals' muscles showed that they burn more fat than do normal mitochondria, but were impaired in their ability to switch to burning glucose. The researchers found that experimentally reducing CrAT activity in laboratory cultures of human muscle cells had similar effects to those observed in mice without CrAT, reducing capacity to switch from using fats to using glucose for cellular fuel. Because people with type 2 diabetes have a defect in fuel switching, the researchers hypothesized that people with the disease may have reduced levels of CrAT in their muscles. Indeed, they found that muscles of people with type 2 diabetes seem to make less CrAT than those of people without the disease. Other experiments suggested that loss of CrAT led to the expected build-up of acetyl-CoA, apparently inhibiting enzymes that are important in glucose metabolism and thus preventing fuel switching to glucose. These observations suggested that it might be possible to help cells of people with type 2 diabetes get rid of the excess acetyl-CoA by providing supplementary carnitine, the nutrient to which CrAT transfers the acetyl group,

and thus making it easier for the enzyme to do its job. The researchers tested this approach in a pilot study in older people with modestly elevated blood glucose levels who were given carnitine supplements for 6 months. Insulin sensitivity improved, and the activity of a cellular enzyme involved in glucose metabolism increased, suggesting that carnitine supplementation may help promote fuel switching to glucose. These results confirm that CrAT is an important regulator in transitioning between glucose and fat metabolism. The preliminary results in the human pilot study, if confirmed through placebo-controlled trials, suggest that carnitine supplementation or other strategies to target CrAT may prove to be viable therapeutic approaches to treatment or prevention of type 2 diabetes.

*Muoio DM, Noland RC, Kovalik JP, et al. Muscle-specific deletion of carnitine acetyltransferase compromises glucose tolerance and metabolic flexibility. Cell Metab 15: 764-777, 2012.*

**Solving a Decades-old Mystery Yields Insight into Metabolic Disease:** After decades of investigation, a transport complex that carries the metabolite pyruvate from the cytoplasm into the mitochondria has been identified. Pyruvate is a key intermediate in carbohydrate, fat, and amino acid metabolism. It is usually produced in the cytoplasm, and then is transported into the mitochondria—the "powerhouses" of the cell—where it is metabolized further. How pyruvate gains entrance into the mitochondria has remained a mystery. Solving this mystery has important implications because altered metabolism underlies common diseases, such as type 2 diabetes and obesity, and, as shown in this study, a less common but devastating disease caused by impaired mitochondrial pyruvate metabolism.

Using an elegant series of genetics, metabolomics, and other analyses, scientists have identified two related proteins, mitochondrial pyruvate carrier 1 (MPC1) and mitochondrial pyruvate carrier 2 (MPC2), that form a complex that transports pyruvate from the cytoplasm into the mitochondria. The genes *Mpc1* and *Mpc2* were initially identified as part of an ongoing effort to characterize mitochondrial proteins that are conserved through evolution. Both yeast and fly mutants lacking the *Mpc1* gene showed reduced pyruvate metabolism; under certain conditions this resulted in poor growth or lethality. Experiments in yeast suggested that the defect was due to diminished entry of pyruvate into mitochondria, thus implicating MPC1 as a pyruvate carrier. Further evidence of MPC1's role in pyruvate transport was provided by studying a chemical, UK-5099, that has been used experimentally to block the activity of pyruvate transport into mitochondria since 1975—even though scientists had not known the exact target of its effects. When assessed in this study, UK-5099 was shown to be a potent inhibitor of MPC1-mediated pyruvate transport in yeast. Further studies in yeast designed to evaluate the interaction between MPC1 and MPC2 suggest that these proteins form a multimeric complex (more than one copy of each protein present) in the mitochondrial membrane. Building on the findings in yeast and flies, the researchers next examined whether MPC1 and MPC2 are involved in transport of pyruvate in mammals. In mouse cells in laboratory culture, turning off the *Mpc1* or *Mpc2* gene impaired pyruvate metabolism. In addition, genetics studies in humans showed that in three unrelated families, children with a devastating disease caused by impaired mitochondrial pyruvate metabolism had mutations in *Mpc1*. Thus, the findings suggest that MPC1 and MPC2 are also serving as pyruvate carriers in humans, and give insight into a serious genetic disease.

The findings of this study solve a mystery that has perplexed scientists working in this area for many years. Moving forward, the identification of these pyruvate carrier proteins opens up new research directions that could be pursued for treating a variety of metabolic diseases.

*Bricker DK, Taylor EB, Schell JC, et al. A mitochondrial pyruvate carrier required for pyruvate uptake in yeast, Drosophila, and humans. Science 337: 96-100, 2012.*

**Fat Cell Gene May Protect Against Type 2 Diabetes:** New research describes how adipose (fat) tissue can protect the body from type 2 diabetes by influencing systemic insulin sensitivity. Insulin, a hormone produced in the

pancreas, stimulates the uptake of glucose in multiple tissues, providing fuel for cells. Adipocytes (fat cells) are sensitive to fluctuations in nutrient availability; they are able to sense and respond to changes in glucose levels. For example, in response to insulin and high levels of glucose, glucose uptake is stimulated in adipose tissue by the transporter protein GLUT4. In obesity, however, GLUT4's activity is reduced in adipose tissue, blocking glucose from entering the fat cells and leading to adverse whole-body metabolic effects, including insulin resistance. How this altered adipose tissue glucose metabolism causes whole-body insulin resistance hasremained a key question.

Knowing that GLUT4 is central to regulation of adipose tissue metabolism, the researchers analyzed changes in the levels of gene activity in mice that were genetically engineered to have high levels of GLUT4 in adipose tissue or to lack GLUT4 specifically in that tissue. This approach enabled the scientists to identify factors involved in the adipocyte glucose response. They observed that a set of genes involved in producing fats was upregulated in mice with high levels of adipose GLUT4 and downregulated in mice lacking adipose GLUT4. High levels of these fat-producing genes in fat cells were previously shown to be associated with the metabolic benefits of enhanced glucose tolerance and insulin sensitivity. Mice with high levels of adipose GLUT4 displayed both of these characteristics, despite being obese. The scientists discovered that, in the mice, the enhanced glucose tolerance and insulin sensitivity required a factor called ChREBP.

To extend their findings to humans, the researchers looked at levels of expression—gene activity—of the *ChREBP* and *GLUT4* genes and insulin sensitivity in over a hundred individuals without diabetes (with normal glucose levels) and with widely ranging body mass index values. They found that adipose levels of *ChREBP* expression correlated strongly with *GLUT4* expression levels and insulin sensitivity, consistent with a role for ChREBP in GLUT4-mediated glucose metabolism. Because not all obese people are insulin resistant, the investigators looked at *ChREBP* levels and insulin-stimulated glucose uptake in obese individuals with widely ranging insulin sensitivity. They observed that *ChREBP* was strongly correlated with insulin sensitivity, suggesting that ChREBP could be a protective factor against obesity-associated insulin resistance. Additional experiments to understand the mechanism of this protection identified a novel form of ChREBP, called ChREBP-β, and higher levels of this form specifically predicted insulin sensitivity in humans.

These results indicate the importance of ChREBP in regulating adipocyte and whole-body glucose control and insulin sensitivity, showing that some features of fat cells can play a critical role in protecting the body against type 2 diabetes. Selective activation of ChREBP-β in adipocytes could be a new therapeutic strategy for preventing and treating type 2 diabetes and obesity-related metabolic diseases.

*Herman MA, Peroni OD, Villoria J, et al. A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism. Nature 484: 333-338, 2012.*

**Suppression of Fat Tissue Inflammation Promotes Insulin Sensitivity:** Scientists have uncovered a key factor in the link between obesity and type 2 diabetes. Insulin resistance is a condition in which the body produces the hormone insulin but does not use it properly. Because this condition leads to increased risk for type 2 diabetes and heart disease, understanding how insulin resistance develops is critical toward efforts to prevent or reverse it. During excess weight gain, a type of immune cell migrates into and accumulates in adipose (fat) tissue and promotes chronic, low-grade inflammation, which contributes to the development of insulin resistance. Another type of immune cell, the regulatory T cell (Treg), is abundant in the adipose tissue of lean, but not overweight, mice and humans. The presence of Tregs in adipose tissue helps to protect mice from developing insulin resistance. However, the molecular mechanisms that regulate the Treg cell population in adipose tissue remained undefined.

In a recent study, researchers found that a protein known to be essential for fat cell development is also critical for maintaining adipose tissue Treg cell numbers in mice.

The protein, called PPAR-γ, resides within adipose tissue Tregs, as well as within fat cells themselves. PPAR-γ controls the activities of different sets of genes, depending on the particular cell type. The scientists genetically engineered mice to lack PPAR-γ exclusively in Tregs, and observed a significant reduction in Treg cell numbers within adipose tissue. Pioglitazone, an insulin-sensitizing drug used to treat type 2 diabetes, is known to activate PPAR-γ. The researchers thus sought to understand whether PPAR-γ in Tregs was responsible for pioglitazone's insulin-sensitizing effects. To test this idea, the scientists treated obese mice with pioglitazone, and observed an increase in Treg cells in adipose tissue, suggesting that the drug treatment can influence the abundance of adipose tissue Treg cells. In these mice, pioglitazone treatment improved metabolic traits such as insulin resistance, glucose tolerance, and insulin tolerance. However, in mice that lacked PPAR-γ in Treg cells, pioglitazone did not increase the abundance of adipose tissue Tregs and improved metabolic traits were not observed. These findings reveal a new role for PPAR-γ in suppressing adipose tissue inflammation by recruiting Tregs to adipose tissue. This study also defines a new molecular pathway for pioglitazone action—one that might be exploited to develop new and effective therapeutics for type 2 diabetes.

*Cipolletta D, Feuerer M, Li A, et al. PPAR-γ is a major driver of the accumulation and phenotype of adipose tissue $T_{reg}$ cells. Nature 486: 549-553, 2012.*

**Molecular Insights Could Lead to New Drugs To Extend Healthy Lifespan:** Researchers have identified a molecular mechanism that could spur the development of new drugs that may lengthen healthy lifespan. A few years ago, the drug rapamycin was found to extend the lifespan of mice. However, rapamycin treatment—though U.S. Food and Drug Administration-approved for use in preventing rejection of transplanted organs and other applications—also comes with significant side effects, including increased susceptibility to infection (because it suppresses the immune system), toxicity to certain organs, and—surprisingly—elevation of blood glucose levels, especially after meals. Rapamycin inhibits a protein called mTOR, which integrates signals from nutrients and growth factors. In new research, scientists sought to understand the molecular mechanisms by which rapamycin extends lifespan yet elevates blood glucose levels. They found that in mice, rapamycin reduced the activity of two different mTOR-containing protein complexes, designated mTORC1 and mTORC2. Using genetically engineered mice to tease out the effects of the different mTOR complexes, they found that the mechanisms by which rapamycin is affecting lifespan and glucose levels are independent: glucose elevation resulted from the mTORC2 inhibition, while the lifespan extension was an effect of inhibiting mTORC1. These findings may pave the way toward the development of new therapies to extend healthy lifespan by targeting mTORC1, while avoiding some of the unwanted side effects associated with rapamycin.

*Lamming DW, Ye L, Katajisto P, et al. Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. Science 335: 1638-1643, 2012.*

## TREATING HYPOTHYROIDISM

**Alternate Therapy May Benefit Some People with Hypothyroidism:** New research has found that some patients who continue to experience symptoms of hypothyroidism despite receiving conventional therapy may benefit from administration of a synthetic form of the thyroid hormone triiodothyronine ($T_3$). Hypothyroidism is a disorder that occurs when the thyroid gland does not make enough $T_3$ to meet the body's needs. $T_3$ regulates metabolism—the way the body uses energy— and affects nearly every organ in the body. Without enough of the hormone, many of the body's functions slow down. The disorder—which can stem from a variety of causes—affects about 4.6 percent of the U.S. population age 12 and older. Symptoms of hypothyroidism include fatigue, weight gain, facial "puffiness," intolerance of cold, and many other problems. The thyroid secretes $T_3$ along with thyroxine, also known as $T_4$, a less potent form of thyroid hormone, which is converted to $T_3$ by various tissues throughout the body. Because $T_4$ is more stable than $T_3$, and the body can generate $T_3$

from $T_4$, current standard therapy for hypothyroidism is a once-daily pill containing a synthetic form of $T_4$.

However, experiments have shown that not all tissues in hypothyroid mice dosed with $T_4$ achieve as high a level of $T_3$ as they need, which may explain why a subset of people with the disorder continue to experience some of its symptoms even when receiving what should be an adequate dose of $T_4$. Previous studies have tested $T_3/T_4$ combination therapy, but the results of these studies have been inconsistent. The present study compared therapy with $T_4$ alone to therapy with $T_3$ alone in 14 participants who had continued to experience some symptoms of hypothyroidism while receiving standard therapy. The study was designed so that after 6 weeks those receiving $T_3$ were switched to $T_4$, and vice versa—with neither the patient volunteers nor the researchers knowing until after the trial was over which participants received $T_3$ in the first half of the trial, and which received it second. Participants took the medicines orally, three times per day, before meals. The researchers found that participants lost weight on $T_3$ relative to $T_4$, showed significant improvement in their levels of cholesterol and other blood fats, and experienced no serious side effects. A longer, larger trial will be necessary to determine the long-term safety and efficacy of this approach. However, this preliminary study suggests that while $T_4$ remains an excellent approach for most people with hypothyroidism, particularly given the relative convenience of its once-daily dosing, thrice-daily dosing of $T_3$ might be a good alternative for those who continue to experience symptoms.

*Celi FS, Zemskova M, Linderman JD, et al. Metabolic effects of liothyronine therapy in hypothyroidism: a randomized, double-blind, crossover trial of liothyronine versus levothyroxine. J Clin Endocrinol Metab 96: 3466-3474, 2011.*

## CYSTIC FIBROSIS RESEARCH

**Research Identifies Key Hurdle in Quest for Cystic Fibrosis Treatment:** Two recent studies have provided a key insight on what has been a puzzling roadblock in attempts to develop a new therapy for the majority of people with cystic fibrosis (CF). CF is an inherited disease of the glands that make mucus and sweat, with serious consequences for the lungs, pancreas, liver, intestines, sinuses, and sex organs. Thanks to the discovery of new antibiotics and improved symptom treatment, the average life expectancy for a CF patient has nearly quadrupled from about 10 years in the 1960s to about 37 years today. Indeed, some people who have CF are living into their 40s, 50s, or older. Despite these gains, life expectancy for CF patients remains much lower compared to healthy adults. CF treatment regimens can be arduous, and managing the disease can be a great challenge for patients and their families.

The development and 2012 approval of a new drug, ivacaftor, has therefore been a tremendous boon to the roughly 5 percent of people with CF who have at least 1 copy of a mutation designated *G551D* in *CFTR*, the cystic fibrosis gene. Ivacaftor was developed through a search for compounds that help stabilize the *G551D* version of the CFTR protein, allowing the protein to reach the cell surface and do its job. In patients with *G551D*, the effect of ivacaftor is to substantially alleviate many of the most serious CF symptoms. Unfortunately, researchers have not yet been successful in finding a compound that can provide a similar benefit to patients with the most common CF mutation, designated *ΔF508*. (Although many CFTR mutations have been identified, about 90 percent of people with CF have at least 1 copy of *ΔF508*.) Two recent studies identify the likely reason why an ivacaftor-like approach—identification of compounds that help stabilize the *ΔF508* form of the CFTR protein—has yet to benefit patients with this mutation. CFTR is a large protein with several "domains," *i.e.*, sections of the protein that "fold" into specific three-dimensional structures with distinct functions. The *ΔF508* mutation changes a part of the CFTR protein called the first nucleotide binding domain (NBD1), eliminating a single amino acid that was previously known to be essential for proper NBD1 folding and function. The new findings show the missing amino acid also plays a key role in interaction of NBD1 with an adjacent CFTR domain, designated the 4$^{th}$ intracellular loop (ICL4). Using different methods, the two groups of researchers reached the same conclusion: that proper

CFTR folding and function require restoration not only of proper NBD1 folding, but also stabilization of the NBD1-ICL4 interaction. Drug discovery screens to date have focused on improved NBD1 folding and function, and have not focused on improving the interaction with ICL4. Now the search is on for a drug or drug combination which corrects both of the *ΔF508* structural issues. If that search is successful, the resulting treatment may one day restore significant CFTR function to people with this CF mutation, potentially reducing the burden of the disease for most people with CF, and allowing them to lead longer, healthier lives.

*Rabeh WM, Bossard F, Xu H, et al. Correction of both NBD1 energetics and domain interface is required to restore ΔF508 CFTR folding and function. Cell 148: 150-163, 2012.*

*Mendoza JL, Schmidt A, Li Q, et al. Requirements for efficient correction of ΔF508 CFTR revealed by analyses of evolved sequences. Cell 148: 164-174, 2012.*

**Researchers Ferret Out a New Model for Cystic Fibrosis-related Diabetes:** The recently developed ferret model of cystic fibrosis (CF) is providing key insights into the development of an important consequence of the disease in humans—CF-related diabetes (CFRD), which is associated with worsening lung function, dangerous weight loss, and increased mortality. Improved antibiotics and other therapeutics have dramatically increased life expectancy for people with CF, roughly quadrupling it over the last 3 decades. As this has happened, complications like CFRD—which affects almost half of CF patients over the age of 30—are of increasing concern to people with CF and those helping them manage their disease. Although the physiological reasons why CF often leads to CFRD are unknown, researchers have found it to be quite different from other major forms of diabetes. Unlike type 1 diabetes, there is no autoimmune attack on the insulin-producing β cells of the pancreas in CFRD. Insulin resistance—a central feature of type 2 diabetes—is often observed to some degree in CFRD, but while obesity is a risk factor for type 2 diabetes, underweight is a much more likely concern in CFRD.

Mouse and pig models of CF have helped clarify what goes wrong in some of the many organ systems affected by the disease. But the utility of these models has been limited by differences between the animals and humans in the way CF affects certain organs and tissues. Of particular note, CF results in enormous damage to the pancreases of pigs even before birth, while CF mice rarely (if ever) develop CFRD. In contrast, CF ferrets have pancreatic problems that more closely mimic human CF, so researchers used that animal model to help uncover the underlying causes of CFRD. They discovered that CF ferrets had early and gradually worsening regulation of glucose levels, not unlike what is often observed in people who have CF. The researchers found that the CF ferrets have smaller clusters ("islets") of β cells at birth, and fewer islets at death, than do control animals without CF. Intriguingly, they also discovered that laboratory cultures of islets from newborn CF ferrets did not respond appropriately to changes in glucose levels: compared to islets from ferrets without the disease, the CF ferret's β cells secreted less insulin at high glucose levels, and also secreted more insulin at low glucose levels. The net effect observed in living CF ferrets was relatively even insulin levels that do not fluctuate normally in response to rising or falling blood glucose levels. These observations show that even at birth, insulin secretion by the pancreas is abnormal in animals with CF. Taken together, the results suggest that despite the insulin resistance often observed in CFRD, the disease quite likely stems from defects in β cell function, and that while some of these β cell problems are present from birth, they are exacerbated by progressive damage to the pancreas. Further study of the ferret model of CF may help researchers find improved ways to prevent or treat CFRD in people with CF.

*Olivier AK, Yi Y, Sun X, et al. Abnormal endocrine pancreas function at birth in cystic fibrosis ferrets. J Clin Invest 122: 3755-3768, 2012.*

## A TIME TO SLEEP AND A TIME TO METABOLIZE

*New discoveries about the relationships between metabolism and circadian rhythms provide possible new approaches to averting conditions such as obesity and diabetes.*

Why does a rapid change in time zone or seasonally setting clocks forward or back throw people for such a loop? We can chalk this up to internal timepieces. In animals and humans, biological "circadian clocks" regulate behaviors and bodily processes—including sleep/wake cycles, changes in blood pressure, and body temperature fluctuations—to harmonize these activities with daily, rhythmic changes in the environment, most notably day/night cycles. One commonly observed sign of the strong influence of circadian rhythms is jet lag, the sleep disturbances and other symptoms that occur after flying across multiple time zones. However, disrupting circadian rhythms can have more insidious consequences.

In humans, misaligning normal circadian rhythms with behaviors such as sleep and eating—for example, by working the night shift—increases vulnerability to diabetes, obesity, and other metabolic problems. One likely reason for these problems is the fact that the circadian clock has a critical relationship with metabolic pathways important to maintaining normal energy balance. For example, the synthesis of glucose (sugar) and fats and the release of glucose into the blood by the liver are governed by the circadian clock. Understanding how circadian rhythm and metabolism are linked therefore could help in the design of strategies to reduce vulnerability to metabolic diseases, and is an area of intense investigation.

Researchers working on the link between the circadian clock and metabolism are actually faced with an additional layer of complexity. Animals and humans possess two types of clocks—a "core" circadian clock, located in the brain, and "peripheral," tissue-specific circadian clocks. The core or master clock responds to cues such as light and dark, nutrient uptake, and temperature. For this clock, a set of core genes has been identified. Some of these clock genes encode activator proteins that help "turn on" target genes, and some of them encode repressor proteins that help "turn off" target genes. Cleverly, the clock genes interact to generate cyclical oscillations in the levels of proteins they encode—*e.g.*, products of activator genes "turn on" repressor genes, and products of repressor genes "turn off" activator genes, so that levels of activator and repressor proteins wax and wane. This leads to downstream effects that occur with regularity within each 24-hour period. The master clock also synchronizes the tissue-specific clocks, which use much of the same genetic machinery. However, the clocks present in each cell can also act and respond on their own, setting up local, tissue-specific rhythms governing gene activation or repression, and subsequent cellular processes—including metabolic activities.

Intriguingly, researchers have found that some of the factors regulating tissue-specific clocks are shared with pathways regulating metabolism. But is there a single factor (or factors) that acts as a key molecular link between circadian rhythms and metabolism? The Rev-erb-α protein has emerged as a candidate. Rev-erb-α is a nuclear receptor protein, meaning that one part of the protein functions as a sensor for a specific signal molecule, or ligand, while another part of the protein functions to bind to DNA in the cell's nucleus and regulate gene activation. The ligand for Rev-erb-α is heme, a small molecule that is integral to many metabolic pathways and whose cellular levels oscillate in a circadian manner. When bound by heme, Rev-erb-α binds to specific target DNA sequences to repress activation of nearby genes. In the context of the circadian clock, Rev-erb-α is believed to regulate how much protein is made by a master clock gene called *Bmal1*. In the context of metabolism, Rev-erb-α regulates genes involved in glucose metabolism, regulates lipid and bile acid production in the liver, and is necessary for the maturation of fat cells from precursor cells. But, because mice genetically

engineered to lack Rev-erb-α do not display a strongly disrupted circadian rhythm, a question has remained as to whether Rev-erb-α plays an accessory role in the circadian clock or is truly a central factor that could link the clock with metabolism.

A recent report has helped to clarify the role of Rev-erb-α. Through a series of experiments in mice, researchers found evidence suggesting Rev-erb-α doesn't just regulate the *Bmal1* gene, but acts cooperatively with the BMAL1 protein at numerous DNA target sites in the liver to regulate the activity of both metabolic and circadian clock genes. Moreover, they found that there is overlap in activity between Rev-erb-α and the closely related protein Rev-erb-β, which could explain why lacking only Rev-erb-α doesn't result in disturbed circadian rhythms. To test this idea further, the researchers used a wheel-running behavior test, a standard method to ascertain circadian dysfunction in mice. In this test, mice first have their rest and active periods artificially synchronized to a cycle of 12 hours of light alternating with 12 hours of darkness. During this initial step, baseline measures of wheel-running rhythms are established; unlike humans, mice are nocturnal and will normally show the most activity during the dark. Then, the mice are put in constant darkness, and their wheel-running behavior is assessed for changes. In the absence of the alternating light/dark cues, normal mice will maintain a circadian rhythm of rest and activity, although the total cycle length will shrink to slightly less than 24 hours. When shifted to constant darkness, mice lacking only one Rev-erb protein showed little or no change in wheel-running behavior compared to normal mice, although mice lacking Rev-erb-α experienced a further shortening of the cycle length. In contrast, mice lacking both the Rev-erbs throughout their bodies showed weak synchronization during the light/dark cycle and, when put into total darkness, decreased and severely fragmented activity and other features indicating circadian dysfunction. As these features are also found in mice lacking BMAL1, their findings suggest both that the Rev-erbs can compensate for each other and that there is a much more central role for Rev-erbs in rhythmic behaviors.

The increasing evidence that Rev-erbs play a central role in both circadian rhythm and metabolism makes them promising therapeutic targets. Knowing that the natural ligand for Rev-erbs is heme, scientists recently synthesized and tested two small molecules for their ability to stimulate Rev-erb effects on circadian rhythms and metabolic outputs in mice. When administered to mice, these molecules were able to repress Rev-erb-responsive metabolic genes in the liver and the oscillation of a core circadian clock gene in the brain. Interestingly, when subjected to the wheel-running behavior test, mice that received single injections of either of the two drugs during the total darkness phase experienced transient but drastic disruptions in running behavior. However, the drugs were much less disruptive when tested under normal light/dark conditions, resulting in only a delay in activity. Drug administration to normal weight mice caused a loss in fat weight and an increase in metabolic rate without any change in food intake and a decrease in activity levels. This appeared to be due to an increase in the levels of enzymes that burn fat. When administered to mice with diet-induced obesity, the drugs improved their metabolic profile, with much greater weight loss compared to normal weight mice, a drop in triglycerides, and cholesterol levels cut nearly in half. The results in both normal weight and obese mice suggest that the drugs exert their effects through Rev-erbs by modifying genetic programs in a way that leads to increased burning of fatty acids and glucose, improving the metabolic profile.

Another potential therapeutic target that has emerged from the study of circadian rhythm and metabolism is a protein called HDAC3. HDAC3 is an enzyme that causes transitory structural changes along the chromosomes called "histone modifications," which affect gene activation. When HDAC3 is recruited to sites in the genome, genes at those sites tend to be turned "off," and when it is absent, those genes are free to be turned "on." Scientists have studied the activity of HDAC3 in the liver and found that, directed by Rev-erb-α, HDAC3 drives circadian oscillations in the activation of genes controlling fat synthesis in liver cells. Now, a team of researchers has made

another intriguing discovery. They found that while depleting HDAC3 in adult mouse livers leads to potentially harmful accumulation of fat in the liver, the fat is sequestered within little droplets surrounded by protective coatings and the mice actually have better insulin sensitivity than mice with normal levels of HDAC3, without any changes in body weight. By examining molecular pathways in the liver involved in the generation, storage, and burning of fat and in the generation of glucose, the researchers determined a likely mechanism: it appears that the constant, rather than rhythmic, activation of lipid-synthesizing and sequestering genes caused by the absence of HDAC3 redirects precursor molecules away from making glucose and toward the synthesis and storage of fat. These findings uncover a previously unknown means for regulating glucose generation and insulin sensitivity in the liver. By distinguishing liver fat accumulation caused by disruption of circadian control of metabolism from liver fat accumulation caused by diet, the findings also suggest that there are multiple pathways by which the liver can end up storing fat, and their potential for harm—or relative benefit—will need to be considered during therapeutic development and application.

In addition to identifying key molecular aspects of circadian control of metabolism, there is another facet to this problem: can we use the knowledge that there *is* circadian control of metabolism to develop behavioral strategies as well as pharmacologic ones to thwart metabolic disease? Researchers recently tested in mice whether timing of feeding, even with a high-fat diet, can influence weight gain and related metabolic problems. They compared four groups of mice: one group was given a normal diet and allowed to eat at any time ("*ad lib*"); another group was given a normal diet, but only during an 8-hour window at night, the natural feeding time for mice ("time-restricted"); a third group was given a high-fat diet *ad lib*; and the fourth group was given a high-fat diet, but time-restricted. At the end of 100 days, the researchers found that, while the mice in all four groups consumed the same number of calories, mice on the time-restricted feeding regimens appeared to have better metabolic profiles. Strikingly, despite having the same diet, mice on the high-fat diet but time-restricted feeding regimen were leaner than their *ad lib* counterparts. At the molecular level, it appears that, by imposing a feeding rhythm, the time-restricted feeding regimen "reprogrammed" activation of pathways governing glucose and fat metabolism in the liver and prevented the circadian and metabolic dysfunction that can occur with a high-fat diet.

As the understanding of the role of factors such as the Rev-erbs and HDAC3 in the circadian control of metabolism continues to evolve, it is helping researchers to test new and existing therapeutic compounds that may help in the fight against diseases such as diabetes and obesity. At the same time, the improved glucose tolerance, protection from obesity, and protection from fatty liver disease seen in mice on a time-restricted, high-fat diet—as well as the metabolic improvements seen in mice on a time-restricted, normal diet—is encouraging, and provides hope that behavioral strategies based on understanding the relationship between circadian rhythms and metabolism can also be developed to prevent metabolic disease.

*Cho H, Zhao X, Hatori M, et al. Regulation of circadian behaviour and metabolism by REV-ERB-α and REV-ERB-β. Nature 485: 123-127, 2012.*

*Hatori M, Vollmers C, Zarrinpar A, et al. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. Cell Metab 15: 848-860, 2012.*

*Solt LA, Wang Y, Banerjee S, et al. Regulation of circadian behaviour and metabolism by synthetic REV-ERB agonists. Nature 485: 62-68, 2012.*

*Sun Z, Miller RA, Patel RT, et al. Hepatic Hdac3 promotes gluconeogenesis by repressing lipid synthesis and sequestration. Nat Med 18: 934-942, 2012.*

# *New Indo-U.S. Collaboration in Diabetes Research*

**On June 12, 2012, U.S. Department of Health and Human Services Secretary Kathleen Sebelius (center) and India's Honorable Ghulam Nabi Azad, Minister of Health and Family Welfare (second from right), signed a joint statement on collaboration on diabetes research. They were witnessed by Dr. Griffin P. Rodgers, Director of the NIDDK (left), Dr. V.M. Katoch, Secretary of India's Department of Health Research and Director-General, Indian Council of Medical Research (second from left), and the Honorable Krishna Tirath, India's Minister of State for Women and Child Development (right). The signing took place at the Hubert H. Humphrey Building in Washington, DC.** ***Photo credit: Chris Smith, HHS***

Diabetes is a global scourge, affecting tens of millions of people around the world. In a move to address this international health challenge, on June 12, 2012, Health and Human Services Secretary Kathleen Sebelius and India's Health and Family Welfare Minister Ghulam Nabi Azad signed a joint statement to begin a formal research relationship in diabetes between the National Institutes of Health and the Indian Council of Medical Research (ICMR). Through this new collaboration, the two nations hope to accelerate efforts to better understand the mechanisms underlying diabetes and to identify innovative solutions to prevent and treat the disease.

About 26 million Americans have diabetes[1]; in India, the burden is estimated at over 62 million people.[2] Millions more in both countries are at increased risk for developing diabetes and its health complications. The challenge of diabetes for the United States and India is complex and multi-faceted: in the United States, persons from racial and ethnic minorities, and those of lower socio-economic status, are disproportionately affected by diabetes. Across India, many challenges exist in accessing affordable health care, including diabetes care. Simultaneously, rapid economic growth and workforce transitions over the last few decades have led to changes in the Indian population's physical activity and diet, which further contribute to diabetes risk. In both nations, diabetes is increasingly striking in younger age groups, with potentially devastating implications for the health, well-being, and productivity of future generations.

In addition to sharing this burgeoning public health problem, both countries already conduct substantial research on diabetes, such as examining lifestyle interventions and metformin to prevent type 2 diabetes. The new joint statement provides greater opportunities for researchers in India and the United States to join forces in projects ranging from research to identify genes for diabetes to bettering public health efforts to manage

and treat diabetes. For example, one potential area of collaboration may be in studying why people of South Asian origin develop diabetes at a lower body mass index and waist circumference than people of other ethnic origins—a question of interest to both India and the United States, with its large South Asian population.

"Both the United States and India have a vested interest in improving our understanding of, and treatment for, diabetes and in finding economical ways to do both," says NIDDK Director Dr. Griffin Rodgers, which will lead the U.S. role in the collaboration. "Initiating this research relationship will enable both countries to share expertise and engage each other in research to lessen the burden of diabetes—in the United States, India, and around the world."

As a first step in partnering, the NIDDK and ICMR held a scientific workshop on February 4-6, 2013, in New Delhi, India. The theme of this initial workshop was the development of affordable and practical approaches and technologies for preventing and managing diabetes and its complications. Both countries could benefit from such approaches and technologies, which are needed to reduce the human toll of diabetes and the high costs of care. The workshop convened diabetes researchers from India and the United States and asked them to identify scientific opportunities in diabetes prevention and management that could be pursued through collaborative efforts. NIDDK and ICMR plan to use these ideas in developing the next steps of the joint Indo-U.S. diabetes research initiative in 2013.

---

[1] *2011 National Diabetes Fact Sheet, Centers for Disease Control and Prevention, Atlanta, GA.*

[2] *Anjana RM, et al. Diabetologia 54 :3022-7, 2012. Epub 2011 Sep 30.*

# STORY OF DISCOVERY

## *A Dramatic Improvement in Care for Some People with Cystic Fibrosis*

A recent breakthrough means that for the first time, some people with cystic fibrosis (CF) can lead their lives with greatly reduced symptoms of the disease. CF is an inherited disease affecting numerous organs throughout the body, including the lungs, pancreas, and intestines. Thick mucus in the lungs of people with CF promotes infections by *Pseudomonas aeruginosa* bacteria, which thrive in the mucus and gradually damage lung tissue. Digestive and pancreatic manifestations of the disease lead to delayed growth and malnutrition. While research has led to enormous strides in CF treatment during the last few decades, dramatically increasing life expectancy for those with the disease, treatments can be arduous and time-consuming, and those with CF remain highly susceptible to dangerous infections and other serious complications. The breakthrough—development of a new medication that can overcome the fundamental molecular flaw in people with a particular mutation of the CF gene—stems from decades of research.

**A Big Advance from Sweating the Small Stuff: The Genetic Cause of CF**

The new treatment has its roots in some of the earliest characterization of the disease. One characteristic of CF that may at first glance seem strangely unconnected to its profound lung, pancreatic, and other consequences is that people with the disease have particularly salty perspiration. Indeed, for many years CF was typically (and quite accurately) diagnosed with a sweat test. Salt is a key ingredient in perspiration, enabling sweat glands to release water in response to increases in body temperature. Evaporation of the water helps cool the body, but the salt—after it has done its job of helping move water out of sweat glands—is no longer needed on the skin. In fact, because the body needs salt (for many purposes in addition to producing more sweat), sweat glands normally reabsorb a portion of what they have released. The first clue to the function of the CF gene came in 1983, with the discovery that the salty skin of people with CF is caused by a defect in this reabsorption process. Specifically, the scientists found that sweat glands from CF patients are much less capable of absorbing chloride—one of the chemical components of salt—than are normal sweat glands. This finding suggested that the genetic mutation underlying CF results in the inactivation of a chloride transport protein.

But more dramatic progress was not possible until the gene encoding that chloride transporter was discovered. Through painstaking genetic analysis, NIDDK-funded investigator Dr. Lap-Chee Tsui and colleagues had identified a region of human chromosome 7 as the likely location of the CF gene. Using the standard gene discovery methods of the era, it might have taken decades to find the CF gene within this region, but Tsui recruited Dr. Francis Collins and his group to join in the effort. Dr. Collins—now the director of the NIH—had recently published a method for making jumps across difficult-to-analyze sections of DNA. This "chromosome jumping" approach dramatically hastened the search. Although it was still a mammoth undertaking, the two groups of investigators announced in 1989 that working together they had succeeded in identifying the gene that is mutated in CF. Confirmation might have been simpler if DNA samples from some people with CF had a large deletion, chromosome rearrangement, or at least

a mutation that would radically change or eliminate the gene, but that did not turn out to be the case. Instead, combing through the genetic information of the gene, they found that just over two-thirds of the chromosome 7s they tested from people with CF (each of whom has two chromosome 7s, one from each parent) were missing the code for just one of the protein's predicted 1,480 amino acid building blocks. Subtle though this tiny deletion seemed to be, they found that most people with CF had two copies of the gene with the deletion, and most of their parents had one copy with the deletion and one without it. Significantly, none of the parents had two copies with the deletion. This was very strong evidence that the researchers did indeed have the correct CF gene, and that the single amino acid deletion was the most common disease-causing mutation.

This signal discovery made it possible to deduce some basic characteristics of the encoded protein, including tell-tale motifs that suggest it functions in cell membranes, and binds a molecule called ATP, which is key for many cellular processes. The arrangement of these motifs was similar to those of a previously characterized protein involved in the transport of substances out of cells. Putting this together with the observations about salty sweat, the researchers correctly guessed that they were characterizing a protein involved in the transport of salt. The gene's discoverers dubbed it the "cystic fibrosis transmembrane conductance regulator" (CFTR). We now know that when the protein binds ATP, it opens a pore on the cell surface through which negatively charged chloride ions (one of the two chemical components of table salt) can travel. The positively charged sodium ions of salt then follow in other ways, keeping the electrical charge balanced in cells. Importantly, this ion flow has the key effect of drawing water along via osmosis, to balance salt concentrations. The flow of water out of lung cells, made possible by movement of chloride ions through healthy CFTR proteins, hydrates the thin, protective layer of mucus on their surface. In people with CF, neither salt nor water flow—so what should be thin, protective mucus becomes thick, sticky, and an ideal habitat for lung-damaging bacteria.

**Progress from Studying CFTR Mutations**

The mutation the researchers discovered that causes elimination of a single amino acid was dubbed *ΔF508* (because it deletes the 508$^{th}$ amino acid in CFTR, a phenylalanine, designated F), and was later shown to represent about two-thirds of CFTR mutations worldwide. Thus, about 90 percent of people with CF have at least one copy of *ΔF508*. (Among people of European descent, about 1 person in 30 has a single copy of *ΔF508*, along with a normal working copy of the CFTR gene, which is enough to avoid CF. Some evidence suggests that CFTR mutations are as common as they are because people with one mutated and one normal version were historically less likely to succumb to diseases like typhoid fever, tuberculosis, or cholera.) Although *ΔF508* is by far the most common mutation, about half of people with CF have at least one copy of one of the thousands of other known CFTR mutations. By studying these various CF-causing mutations, researchers have discovered a great deal about the CFTR protein, its function, the disease physiology of CF, and—ultimately—medicinal approaches to address those problems at the molecular level. For example, they found that *ΔF508* results in a protein that is unstable, and that is degraded before it gets to the cell membrane—which helped clarify the maturation process by which the cell prepares the protein for its role on the cell surface. Other mutations, such as one designated *G551D*, result in CFTR proteins that reach the cell surface in adequate quantities, but which fail to open and therefore do not allow the flow of chloride through the CFTR "gate." CF

researchers therefore designated mutations like *G551D* that yield stable but non-functional CFTR proteins to be "gating" mutations. "Conductance" mutations gate normally, but contain a defective pore through which chloride cannot travel. The different types of mutations provide key information about the way the parts of CFTR work together to regulate the flow of chloride.

Intriguingly, researchers found that ΔF508-CFTR proteins could actually reach the cell surface—and even transport chloride—if made by cells grown in cool conditions (well below body temperature) in the laboratory. This suggested the tantalizing possibility that if they could somehow identify medicinal compounds that stabilize ΔF508 at body temperature, or that allow CFTR proteins made with gating or conductance mutations to allow chloride flow, they might effectively be able to treat the fundamental molecular problem in CF.

**Progress from Technological Innovation**

CF researchers therefore sought to identify candidate medicines that could promote CFTR function. A key step in that discovery process was the creation of stable cell lines bearing various human CFTR mutations that could be grown in the laboratory. In principle, investigators could then expose the cells to different candidate drugs, and ask which allowed the transport of chloride. Checking for ion transport, however, is no easy task if it has to be performed one chemical at a time for hundreds of thousands of potential medicines. The work was therefore greatly advanced by the development of fluorescent markers of CFTR activity. For example, one NIDDK-supported group created a protein that fluoresces when exposed to ultraviolet light, but dims substantially when bound to ions like chloride. Another group employed chloride-sensitive-dyes. These innovations allowed the medicine hunters to create large arrays of test chambers containing CFTR-mutant cells, accompanied by a different candidate drug in each chamber, and then use fluorescence to detect ion flow in many chambers at once. Screening hundreds of thousands of compounds, in this fashion, the searchers were able to identify a few that had properties they were looking for—helping different mutant CFTR proteins function better. The promising candidates they discovered were then chemically tweaked in various ways, to try to improve on their ability to promote chloride flow. Of course, these compounds might work beautifully in the laboratory, but to be useful as CF drugs, the compounds would have to be proven safe, and capable of reaching cells where they are needed. Thus, laboratory animals such as mice that have been engineered to have the same CFTR mutations found in humans were another critical resource for preclinical testing.

Among the compounds found to have the most promising qualities was one designated VX-770 by the pharmaceutical company where it was identified. Following animal testing and a preliminary dosage trial in humans, it was eventually shown in trials of increasing size and length to significantly improve function of the lungs, pancreas, and other affected organs in people with at least one copy of the *G551D* gating mutation. Based on these strong safety and efficacy results, the U.S. Food and Drug Administration approved VX-770 (as "ivacaftor," marketed as "Kalydeco™") in January, 2012. This is great news for people with CF in the United States who have at least one copy of the *G551D* mutation. It accounts for about 2 percent of CF-causing mutations in the United States, (meaning about 4 percent of U.S. residents with CF have a copy of the mutation), but is somewhat more common in Ireland, Scotland, Brittany (in France), and the Czech Republic. Several other, rarer gating mutations are also known, and it is hoped the drug

may also prove valuable in patients with some of these forms of CFTR as well.

Although initial characterization of VX-770 and of a few other compounds identified through similar screening approaches suggested they may be of benefit to anyone with the much more common *ΔF508* mutation, this has unfortunately not proved to be the case. A drug does not have to restore complete function to CFTR to be of substantial benefit to people with CF—achieving 10 percent or more of chloride transport activity is expected to have a real impact on symptoms. So why have such efforts so far been unsuccessful in the case of *ΔF508*? Two recent papers are helping provide an explanation: ΔF508 not only interferes with folding of the first ATP binding domain of CFTR, it also disrupts interaction of this domain with a neighboring part of the protein. Because there are two physiological problems with the same protein, it is not enough to correct one of them. In fact, it may be necessary to improve the protein folding, the domain interaction, or both, by quite a bit more than 10 percent to achieve a net restoration of 10 percent of normal channel function. This is a substantially higher hurdle to cross, but the search may be facilitated by this better understanding of what such a drug (or combination of drugs) must achieve. Thus, it may be productive to search for a compound that promotes a significant increase in chloride transport in cells with the *ΔF508* mutation in the presence of VX-770 or another compound, which by itself confers only a modest improvement.

Other drugs are in development for other types of CFTR mutations. For example, the CFTR gene can be thought of as an instruction list for the cell, indicating the order and identity of each of the CFTR protein's 1,480 amino acids. Some mutations change the list, so that the code for a particular amino acid is changed to a code instructing the cell's protein production machinery to stop adding amino acids to that protein. Drugs that can induce the cell to "read through" such "stop" instructions are also in development. Importantly, these ongoing CF drug searches are made possible by the same fundamental advances that enabled the development of VX-770 for people with CF and the *G551D* mutation: discovery of the CFTR gene, characterization of the protein it encodes, analysis of the CF mutations, and the creation of CF cell lines, animal models, and fluorescent ion sensors. Thus, the quest continues for medicines to help more people with CF lead lives that are longer, healthier, and less burdened by the disease.

# SCIENTIFIC PRESENTATION

## Emerging Strategies To Combat β Cell Failure in Diabetes

*Dr. Domenico Accili*

*Dr. Domenico Accili is a Professor of Medicine at Columbia University and Director of the Columbia University Diabetes Research Center in New York City. He is a graduate of the University of Rome and trained in internal medicine at the University Hospital Gemelli in Rome. Following a Fogarty Fellowship in the Diabetes Branch of the NIDDK's Intramural Research Program, he became Chief of the Section on Genetics and Hormone Action at the* Eunice Kennedy Shriver *National Institute of Child Health and Human Development. Since 1999, he has served on the faculty at Columbia University's College of Physicians and Surgeons and as an attending physician at Columbia-Presbyterian Hospital.*

*Dr. Accili has served on several editorial boards; is a member of numerous advisory panels for academia, government, and industry; and his research has been published in leading medical journals. He has received numerous awards, including the 2003 Lilly Award for Outstanding Scientific Achievement by the American Diabetes Association, and is an elected member of the Association of American Physicians and the American Society for Clinical Investigation. His work is supported by the NIH, American Diabetes Association, Russ Berrie Foundation, and Brehm Coalition. Dr. Accili presented research findings from work conducted in his laboratory at the May 2012 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council. The following are highlights from his presentation.*

**Loss of β Cell Function Drives Type 2 Diabetes**

Glucose (a type of sugar) is the fuel of cells, providing energy that enables cells to carry out their various functions. Glucose can be derived from the consumption of food or from production by the liver. The essential hormone insulin, which is produced by the pancreas, stimulates the uptake of glucose from the blood by cells in muscle, fat, and liver, ensuring that these tissues are provided with the necessary fuel. β cells, which are found in the pancreas within cell clusters called islets, are the body's sole source of insulin. β cells carry out key functions in producing insulin and secreting insulin into the blood.

Diabetes is characterized by the body's failure to produce and/or respond appropriately to insulin, and results in the inability of the body to absorb and use glucose. In people with type 2 diabetes, cells do not properly react to insulin; this is referred to as "insulin resistance." As a result, the pancreas initially produces more insulin to compensate. Gradually, however, the β cells lose their ability to secrete enough insulin to restore balance, and the timing of insulin secretion becomes abnormal, causing blood glucose levels to rise. Therefore, type 2 diabetes is characterized as a combination of two abnormalities: impaired insulin action and β cell dysfunction. It has been unclear, however, whether one of these drives the development of the disease more than the other, and whether doctors should focus treatment on one abnormality (and, if so, which one) or both.

# SCIENTIFIC PRESENTATION

Scientists in the NIDDK's Intramural Research Program in Phoenix, AZ conducted a study to determine the sequence with which these abnormalities develop and their relative contributions to loss of maintenance of normal glucose tolerance *(i.e.*, progression to type 2 diabetes). By following a population at high risk of developing type 2 diabetes, the scientists observed that some people were able to maintain normal glucose tolerance, despite deterioration of the response to insulin action, because their insulin production increased. The increased amount of insulin was sufficient to shuttle the excess glucose from the bloodstream. The disease developed, however, in people whose β cells were unable to sustain increased insulin production: loss of β cell function was a key difference between those who progressed to the disease and those who did not. Therefore, impaired β cell function is a driver in the pathogenesis of type 2 diabetes. Understanding and combating β cell failure is thus critical to treating diabetes. Unfortunately, much is still unknown about how and why β cells fail, and treatments to improve β cell function effectively are lacking.

**What Causes β Cell Failure?**

Visualization of the progression to diabetes in rodent models has enabled scientists to document what happens to the pancreatic islet in great detail. Using experimental procedures to tag insulin with a dye visible by microscopy, they were able to mark β cells that are producing insulin and thus have not failed. Early in the course of the disease, when glucose levels are still normal and cells are developing resistance to insulin, scientists observed that, in the rodent model, the mass of β cells expands in response to insulin resistance. This is followed, as the disease progresses, by a decrease in β cell mass and loss of insulin. Dr. Accili and his colleagues hypothesize that human islets undergo a similar process in the development of diabetes, but this has not yet been demonstrated. It has been suggested that the decrease in β cell mass results from the death of β cells, and there are numerous theories as to the cause(s) of β cell death. Dr. Accili noted that these proposed causes likely occur in β cells, but speculated that none of them is specific to the β cells of a person with diabetes, suggesting that none of the proposed causes is likely to be the driver of β cell failure in diabetes.

**FOXO: An Important Sensor in Mammalian Metabolism**

Dr. Accili's efforts to elucidate the driver of β cell failure has focused on a family of proteins—called FOXO—that regulates the levels of genes involved in a diversity of cellular processes, including the response to DNA damage, cell death, cell proliferation, stress tolerance, and longevity. Interestingly, FOXO proteins have also been implicated in the cell's response to insulin and metabolism. The link between FOXO proteins and insulin came to light in research from another laboratory studying the roundworm *C. elegans*. Scientists observed that modifying worms so that they lack the gene for the insulin receptor, a protein that is required for the function of insulin, led to accumulation of lipids (fat) in the intestines. The scientists were able to visualize the presence of intraintestinal lipids using a dye. This enabled them to screen through a collection of worms that each had a different second mutation—in addition to the original genetic modification—for one that reversed the accumulation of intraintestinal lipids. They identified the *C. elegans* relative of the mammalian FOXO proteins, providing the first clue that FOXO is involved in the response to insulin.

Studies from Dr. Accili's laboratory and others showed that one specific FOXO protein—called FoxO1—integrates signals regulating β cell mass and stress response and responds differently to

insulin and glucose. In most cell lines, FoxO1 can predominantly be found within the nucleus, the compartment containing the cell's DNA. In response to insulin, FoxO1 is quickly removed from the nucleus and inactivated as a result of its translocation to the area of a cell called the cytoplasm. In response to glucose, FoxO1 takes the opposite journey; any FoxO1 found in the cytoplasm is concentrated in the nucleus and localizes in a specific pattern. This shuttling of FoxO1 in response to hormones and nutrients allows it to be an effective sensor that relays the nutritional and hormonal status of an organ to the nucleus of a mammalian cell, enabling FoxO1 to alter which genes are turned on and off by controlling DNA transcription.

**The Role of FOXO in β Cell Function**

Because FoxO1 had characteristics of an important metabolic sensor, Dr. Accili was specifically interested in what happens to FoxO1 during development of diabetes. He and his colleagues took advantage of techniques that allowed them to visualize FoxO1 and insulin in the islets of mouse models of diabetes. First, they observed that FoxO1 was found only in β cells, not in any of the other cells found in islets. In healthy β cells, with normal glucose levels, FoxO1 and insulin co-localized to the cytoplasm of the cell. Because they observed FoxO1 in the cytoplasm, this indicated that it was inactive in healthy β cells. In early diabetes, with mild increases in the level of glucose, they found that FoxO1 relocated to the distinctive nuclear pattern in response to the stress, and insulin was less visible than it was in healthy β cells. As diabetes progressed and levels of glucose increased, both insulin and FoxO1 became even less visible, indicating that there is a tight correlation between progression of β cell dysfunction and levels of FoxO1. They did not know, however, whether the loss of FoxO1 was a cause or an effect of β cell failure, nor what happened to the cells that were producing insulin.

To learn more about the role of FoxO1 in β cell failure, Dr. Accili and his colleagues genetically modified mice to lack FoxO1 specifically in their β cells (while leaving it intact in other parts of the body). Again, they used techniques to visualize insulin and also glucagon, a hormone produced by the pancreas that signals the liver to release stored glucose. Under standard conditions, mice lacking FoxO1 in their β cells appeared relatively normal. They displayed normal glucose tolerance and insulin and glucagon secretion. To assess the consequences of loss of FoxO1 in the response to metabolic stress, the scientists looked at the FoxO1-deficient β cells of female mice that had undergone multiple pregnancies (multiparous) and of aging male mice. Intriguingly, in these mice, the FoxO1-deficient β cells underwent a similar process as the diabetic β cells, suggesting that loss of FoxO1 could be a cause, rather than a consequence, of β cell dysfunction. Under both of the metabolic stress conditions, they observed increased glucose levels, impaired glucose tolerance, decreased insulin secretion, and increased glucagon secretion. These conditions—elevated glucose and glucagon levels and low insulin—mimic conditions found in humans with type 2 diabetes, suggesting that the β cell dysfunction in FoxO1-deficient mice could be similar to that in people with diabetes.

Because only the β cells had been altered in these mice, the resulting elevated glucose and glucagon levels and low insulin are not due to insulin resistance; cells that respond to the activity of insulin had not been modified. Therefore, the scientists could probe why β cells without FoxO1 failed in the absence of insulin resistance. To determine whether the loss of insulin production was due to death of the β cells or to loss of their capacity to carry out their normal function, Dr. Accili and his colleagues performed a "lineage tracing experiment" where, using the previously

described mice lacking FoxO1 in their β cells, they labeled the β cells without FoxO1 with one color chemical label and insulin-containing cells with another color label. β cells that lacked FoxO1 but could still produce insulin would thus have both color labels. This experiment allowed the scientists to draw conclusions about what happens to the FoxO1-deficient β cells when animals have undergone metabolic stress, such as multiple pregnancies. For example, if the FoxO1-deficient β cells die, they would expect to see no cells labeled with the color that marks FoxO1 deficient cells.

In virgin mice, Dr. Accili and his colleagues observed that FoxO1-deficient and normal islets looked similar, suggesting that, without metabolic stress, mice with FoxO1-deficient β cells have a similar endowment of β cells and that these β cells function properly. Following multiple pregnancies, the islets of the FoxO1-deficient mouse—which had since developed diabetes—looked different from the islets of a multiparous, normal mouse. Unexpectedly, they observed an increase in the number of FoxO1-deficient cells without insulin. Thus, the FoxO1-deficient β cells did not die, but instead lost the ability to produce insulin.

**New Identities for β Cells in the Absence of FOXO**

To understand more fully the β cell failure in these FoxO1-deficient cells, Dr. Accili and his colleagues looked at important markers of β cell identity and function, including factors called MafA and Pdx1. They observed that these markers were absent in the cells of FoxO1-deficient mice that no longer produced insulin. Because these markers are important to β cell development, Dr. Accili and his colleagues wondered if these FoxO1-deficient cells had lost even earlier markers of β cell identity.

They focused on a factor called Neurogenin3 (abbreviated Neurog3), which is found in endocrine precursor cells. Neurog3 is a marker shared in common by cells destined to develop into different endocrine cell types. This is an important stage, therefore, because a cell making Neurog3 has made a decision to become an endocrine cell, but has not yet committed to which endocrine cell type it will become. Because this stage occurs prior to birth in mouse and human, the adult pancreas contains few, if any, cells with Neurog3. In multiparous mice that lack FoxO1 in their β cells, the scientists observed a number of cells with Neurog3. They also observed the appearance of Neurog3-containing cells in aging male mice. Cells with high levels of Neurog3 contained no insulin, Pdx1, and MafA, while normal mature β cells showed the opposite pattern. This important result suggests that a developmental stage—when precursor cells are marked by Neurog3—that was previously thought to be very transient and never reproduced in the life of an adult β cell, occurs when adult β cells lack FoxO1. In addition to Neurog3, other auxiliary markers that normally accompany Neurog3 were present as well. This indicated that FoxO1-deficient β cells revert to an uncommitted precursor-like stage under conditions of metabolic stress.

Another aspect of type 2 diabetes is that people with the disease have high levels of glucagon, so Dr. Accili and his colleagues wondered whether the reversion of FoxO1-deficient β cells to a progenitor-like state was associated with potential for conversion to another pancreatic cell type. When they visualized insulin and other pancreatic hormones (like glucagon) in a normal islet, they did not observe cells that contained both insulin and other hormones known to be made by other types of islet cells. In the absence of FoxO1, however, they observed these other hormones in cells that used to be β cells (before the loss of FoxO1 following exposure

to metabolic stress). This observation suggests that the FoxO1-deficient cells had lost their β cell identity and had taken on a new identity—producing other pancreatic hormones. This provides an explanation for the high levels of glucagon seen in FoxO1-deficient, multiparous mice and suggests that high levels of glucagon seen in people with diabetes might be explained, in part, by cells losing their β cell identity and taking on a new, glucagon-producing identity. None of the converted cells produced insulin as well, consistent with the idea that regression of FoxO1-deficient β cells to a distinctive pre-β cell state is a prerequisite for their conversion into a new identity.

To ask whether these observations were limited to experimentally induced deficiency in FoxO1 or whether they represented a common path to β cell failure, the scientists looked at mice that spontaneously developed diabetes and observed that, although insulin levels decreased as glucose levels increased, the β cells did not die. Rather, in the diabetic mice the β cells became marked by Neurog3 and its accompanying factors and appeared to lose FoxO1. Therefore, it appears that, in mice, FoxO1 is required to enforce β cell identity in insulin-resistant diabetes and suggests that gradual loss of FoxO1 during diabetes progression leads to loss of β cell identity and causes the pathogenic β cell failure observed in type 2 diabetes. Dr. Accili and his colleagues are beginning experiments to determine whether the same process occurs in humans.

**Conclusions and Implications**

Dr. Accili's research indicates that loss of β cell identity, not β cell death, is a key feature of type 2 diabetes. This is a departure from the widely held view that β cell failure is caused by a reduction of β cell mass following cell death. Rather, this research shows that β cells lose their ability to produce insulin and gain a new identity and the ability to produce glucagon, and that this process is caused by loss of FoxO1 function. FoxO1 is required to maintain β cell identity and prevent conversion into other pancreatic cell types in response to chronic metabolic stress. Dr. Accili proposed that FoxO1 carries out these critical functions by promoting genes required for β cell identity and by preventing reactivation of precursor genes.

Dr. Accili suggested that these new results could lead to improved approaches to treat type 2 diabetes. However, the current research emphasis to develop new therapeutics that increase production of insulin from remaining β cells or that induce β cell replication do not address this critical problem. Treatments that salvage cells that have regressed to become progenitor-like cells and restore them to become β cells again could be fruitful, taking advantage of the fact that the cells are still alive and present. Allowing metabolically stressed β cells to rest may be key to such an approach. Clinical studies have suggested that treating newly diagnosed patients aggressively with insulin, either with a pump or multiple daily injections, induces a remission of type 2 diabetes such that normal fasting glucose levels are maintained for some time after insulin is withdrawn. Other diabetes medicines do not achieve such prolonged effects after they are discontinued, and insulin is only able to do so early in the course of type 2 diabetes. In other words, providing insulin to meet the demand on β cells could allow β cells to "rest" and result in a window of opportunity to intervene in the progression of the disease. Dr. Accili put forth that, once ways are devised to coax former β cells back to a β cell identity, coupling this approach with intensive insulin treatment at disease onset could become an improved strategy for treating type 2 diabetes.

*Talchai C, Xuan S, Lin HV, Sussel L, and Accili D. Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. Cell 150: 1223-1234, 2012.*

# PATIENT PROFILE

## Anastasia Albanese-O'Neill

*Research To Combat Type 1 Diabetes: It's All in the Family*


Anastasia Albanese-O'Neill, with husband Dan and children Cassidy and Jackson

In 2002, little did Anastasia Albanese-O'Neill know that her personal and professional life would change because of an unexpected diagnosis. It was that year that her daughter, Cassidy, was diagnosed with type 1 diabetes at 16 months of age.

Type 1 diabetes is an autoimmune disease in which the immune system destroys cells in the pancreas that make insulin. People with the disease must carefully monitor blood sugar levels and administer insulin, either through injections or an insulin pump.

"We didn't know anything about diabetes," recalls Anastasia. "No one else in our family had the disease." Cassidy was diagnosed with type 1 diabetes in the hospital and admitted to the intensive care unit (ICU) because her blood sugar levels were dangerously high. "In the ICU we learned that there was no cure for type 1 diabetes and that Cassidy would need multiple daily insulin injections and 8 to 10 finger pricks every day to monitor blood sugar levels. We were sent home with a box of medical supplies and, to be honest, a sense of dread."

Even under those life-changing circumstances, she and her husband, Dan, recognized the importance of research to combat type 1 diabetes and enrolled Cassidy into a research study. Since that time, the family has continued to make major contributions to type 1 diabetes research.

**Participating in the SEARCH for Diabetes in Youth Study**

"While we were in the hospital, we were asked to participate in a study called SEARCH for Diabetes in Youth," Anastasia remembers. SEARCH is a joint effort of the NIDDK and the Centers for Disease Control and Prevention, and receives support from the *Special Statutory Funding Program for Type 1 Diabetes Research*. SEARCH was started to provide much-needed information on how many children and youth under age 20 in the United States have diabetes and how those rates were changing over time. That information is critical for informing public health efforts to fight the disease.

Although it was daunting for Anastasia and Dan to find out that their young daughter had type 1 diabetes, they were eager to sign up for the SEARCH study. "It was an

# PATIENT PROFILE

easy study to participate in—we just needed to share some of our daughter's medical and demographic information, and so we enrolled," she explains.

And their effort has paid off. Because of SEARCH, which is conducted by researchers at multiple centers around the country, the Federal government now has an estimate of how many children and youth in the United States have diabetes: an estimated 15,600 youth are diagnosed with type 1 diabetes each year and an estimated 215,000 youth had diabetes (type 1 or type 2) in 2010. SEARCH has also found that rates of type 1 diabetes in youth under age 20 have increased by a surprising 23 percent between 2001 and 2009. Exact reasons for the increase are not known, but researchers speculate that there is something in the environment that is triggering the disease. A question remains as to what the environmental trigger, or triggers, may be—knowledge that is critical to finding ways to prevent type 1 diabetes. Anastasia and her family are also helping to answer that important question.

*"I can tell you with absolute certainty that the course of Cassidy's life so far, along with her future prospects, are indisputably better because of medical research," says Anastasia.*

**Participating in the TEDDY Study**

In 2005, Anastasia and Dan welcomed their second child, Jackson. Before Jackson was born, the family found out about another NIDDK-led research study, called The Environmental Determinants of Diabetes in the Young, or TEDDY. This international study was seeking to enroll and follow newborns to identify environmental triggers of type 1 diabetes. Anastasia and Dan allowed researchers to test Jackson's blood shortly after he was born to see if he was at increased genetic risk for developing type 1 diabetes. "Unfortunately, he was," his mom recalls, which made him eligible to participate in TEDDY.

Anastasia and Dan next had to decide whether or not to enroll their son into the study. "My husband and I—after much discussion—agreed to participate," explains Anastasia. "It was a tough choice. The study required a 15-year commitment from our family." Jackson became one of the first children enrolled in TEDDY, and is now one of over 8,000 children being followed until they are 15 years old to collect data on their infectious, dietary, and other exposures and life experiences toward identifying an environmental trigger. TEDDY also is supported by the *Special Statutory Funding Program for Type 1 Diabetes Research.*

Identifying environmental factors is much like looking for a needle in a haystack because there are so many factors that could be the culprit. Thus, "the list of data collected on each child is extensive," explains Anastasia. "We provide samples of our water, we send in toenail clippings, we keep track of what Jackson eats and when he was immunized. We even overnight [mail] stool samples." They've become so used to doing this, "I don't get nervous at the post office when they inevitably ask, 'What's in the box, Ma'am?' If Jackson is along, he answers for me," she says with a laugh.

Jackson has been a real trooper, especially during the blood draws—the toughest part for him. "Each time we go to an appointment, we ask him if he would like to skip the blood draw, but he's only asked not to do it once," Anastasia reports. "He always replies that he needs to do it for Cassidy." She explains that her son experiences the day-to-day challenges of living with type 1 diabetes and sees this study as his way of helping. "I'm really proud of him," says his mom.

# PATIENT PROFILE

TEDDY has the potential to revolutionize the ability to prevent type 1 diabetes. For example, identification of a dietary or infectious cause could have an enormously positive impact on public health through a diet change or vaccine for disease prevention. Therefore, Jackson is helping countless future generations of children who may be spared from type 1 diabetes because of his and his family's dedication to research. "If we can find a way to prevent the disease, it will be worth all the effort," says Anastasia.

**A Career Change to Diabetes Research**

One morning in 2007, Cassidy asked her mom an unexpected question. "I was driving my daughter to school when she was six, and she asked from the back seat, 'Mommy, what are YOU doing to cure diabetes?'," Anastasia recalls. "I don't remember exactly how I responded, but I know that later that week I enrolled in a chemistry class as a prerequisite to nursing school," even though she was working as a marketing director for a community college at the time and had no medical background.

In 2008, Anastasia received her nursing degree, and began working part-time in a research laboratory. During that time, she also had the opportunity to work in a pediatric diabetes clinic as a nurse educator, where she worked with families of children with diabetes. As a parent of a young child with the disease, she was able to relate to those families and felt that she could help them. That feeling prompted her to take her training a step further and enroll in a graduate program focusing on the use of technology in diabetes care and management. "I am working toward becoming a nurse practitioner and getting a Ph.D. in nursing because I want to do research to help families have better technologies," she explains. She is currently pursuing those studies full-time, while continuing to work part-time in the clinic. This is another example of how she is making far-reaching contributions to type 1 diabetes research.

**A Family's Hope Through Research**

Although she is busy raising two young children, participating in an intensive research study, pursuing graduate studies, and working in the clinic, Anastasia makes time to advocate for research on type 1 diabetes. For example, she is serving a 2-year term on the American Diabetes Association's National Advocacy Committee. Why does she do it? "Without research, there is no hope for prevention, for a cure, or for better treatments. For our family, it's simple. Research equals hope," Anastasia exclaims.

She also says that the importance of research is evident when she looks at her daughter. Cassidy started using an insulin pump when she was 2 years old and also uses other devices, such as blood sugar meters that give a near-immediate reading. These technologies allow her to keep her blood sugar at almost normal levels, which is important because research has shown that good blood sugar control greatly reduces risk for the development of long-term diabetes complications. "I can tell you with absolute certainty that the course of Cassidy's life so far, along with her future prospects, are indisputably better because of medical research," says Anastasia.

*"Without research, there is no hope for prevention, for a cure, or for better treatments. For our family, it's simple. Research equals hope," Anastasia exclaims.*

However, that in no way means that managing type 1 diabetes is easy. It is a daily struggle: "We cannot get through a day without having to answer to it—it is unrelenting," says Anastasia. "I think the hardest part

# PATIENT PROFILE

is that diabetes takes away the spontaneity, freedom, and innocence of being a child. If Cassidy is going to a sleepover or a birthday party, we have to have a discussion with the other parents about diabetes. If she is going for a swim, she has to worry about her insulin pump being in the sun and whether her blood sugar will be too high or too low while in the water. As a mom, I want really badly for my child not to have to deal with those kinds of problems and to have the freedom to just be a kid." Those are just a few reasons why finding a cure is so important. "The day that a cure is discovered will be a day to rejoice."

In the meantime, Cassidy and her family are vigilant about managing her type 1 diabetes. And the proud mom is happy to report that, "My daughter is a happy, healthy 11-year-old in the sixth grade, who dances and plays volleyball and does well in school."

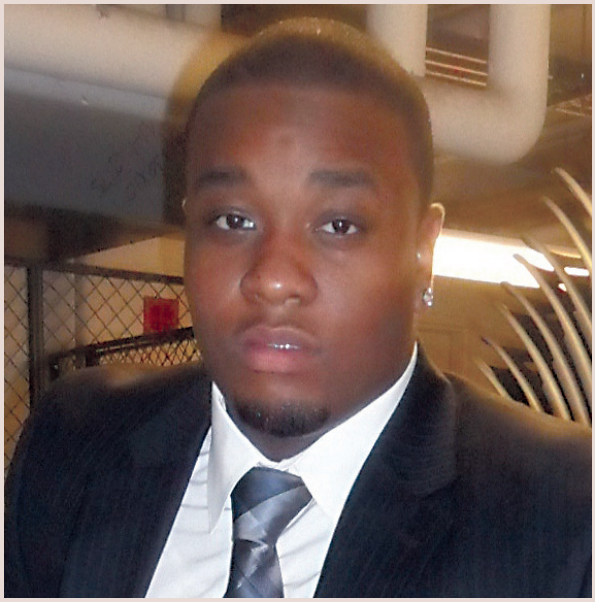
Jackson, 7 years old and in the second grade, is a competitive gymnast and also does well in school. He remains an active participant in the TEDDY study. "The good news is that he does not have diabetes," his mom notes happily.

As for Anastasia, her dedication to type 1 diabetes research is unwavering: "I plan to retire within minutes of the discovery of a cure for type 1 diabetes, but not a moment earlier."

# PATIENT PROFILE

## Jameel Shareef

*TODAY's Lifestyle for Tomorrow's Health with Type 2 Diabetes*



Jameel Shareef

Twenty-year-old Jameel Shareef describes himself as a funny, humorous, spontaneous guy who likes to make people laugh. He loves football, and his life's ambition is to become a football analyst for a major cable news sports network.

Diagnosed with type 2 diabetes at age 13, Jameel knows that if he's going to be successful at achieving his life's dream, he's also going to need to succeed at managing his diabetes.

**Jameel and Type 2 Diabetes**

Currently a college student, Jameel first noticed something wasn't normal when he was in middle school, during an hours-long bus trip to watch a football game at Giants Stadium, near New York City. "During that trip, I was constantly thirsty," says Jameel, "and on the ride home I was using the bus bathroom every 5 or 10 minutes."

Things got progressively worse. "When I started school that September, in 2005, I felt tired and looked pale, my skin complexion started to change, and I constantly was drinking orange juice," he says. His mother reacted quickly and took him to a nearby medical center, where he was immediately diagnosed with type 2 diabetes.

"The same month as my diagnosis, a nurse brought the TODAY (Treatment Options for Type 2 Diabetes in Adolescents and Youth) study to my attention and strongly encouraged me to enroll," says Jameel. "I was told it was good I was diagnosed early so that I can control my diabetes and stay healthy." With his parents' consent and support, Jameel enrolled in the study.

Type 2 diabetes was previously called "adult-onset" diabetes because it was predominantly diagnosed in older individuals. In the mid-late 1990s, however, doctors began increasingly to see type 2 diabetes in adolescents. Over the years since, and believed to be related to the childhood obesity epidemic, there has been a dramatic rise of type 2 diabetes in young people like Jameel. The SEARCH for Diabetes in Youth study, a joint effort of the NIDDK and Centers for Disease Control and Prevention, reported that in the United States, an

# PATIENT PROFILE

estimated 3,600 young people develop type 2 diabetes each year, with youth from racial/ethnic minority groups disproportionately affected.

*"...it was good I was diagnosed early so that I can control my diabetes and stay healthy," says Jameel.*

With the rise of type 2 diabetes in youth comes another serious problem. The longer a person has type 2 diabetes, the greater the likelihood that they will develop complications from the disease, including heart disease, stroke, eye disease, nerve damage, and kidney disease. This makes it critical for young people with type 2 diabetes to achieve quick and sustained control of their blood sugar, which is why Jameel's enrollment in the TODAY study early in the course of his disease was important.

**About the TODAY Study**

Because type 2 diabetes had been primarily an adult illness, information about how to effectively treat young people with the disease was limited, leaving pediatric diabetes experts to rely on what was known about how to treat adults with the disease. The TODAY study set out to determine how best to treat children and adolescents. This study was conducted by researchers at 16 medical centers around the country, and funded by NIDDK. The study enrolled young people who had been diagnosed with type 2 diabetes for less than 2 years, and had a body mass index (BMI) at the 85$^{th}$ percentile or greater. BMI is a measurement of weight in relation to height. In children and adolescents, overweight is defined as a BMI at the 85$^{th}$ to 94$^{th}$ percentile for their age and sex; obesity is defined as a BMI at the 95$^{th}$ percentile or more. At the time of his diagnosis, Jameel was 5 feet 9 inches tall and weighed 234 pounds, which placed his BMI in the obese category for youth, and he was eligible to enroll in the TODAY study.

The study enrolled nearly 700 young people between the ages of 10 and 17, who agreed to participate for 2 to 6 years. Each TODAY study participant was randomly assigned to one of three treatment options: (1) the diabetes medication metformin; (2) metformin plus another drug, rosiglitazone; or (3) metformin plus an intensive lifestyle program that coordinates nutrition, physical activity, and behavior modification. Metformin is a widely used, first-line treatment for adults with type 2 diabetes. Currently, metformin is the only oral drug approved by the U.S. Food and Drug Administration (FDA) for treating type 2 diabetes in youth ages 10 to 17.

In April 2012, TODAY study researchers reported that metformin alone was inadequate for maintaining acceptable, long-term, blood sugar control in over 50 percent of youth. Adding the lifestyle intervention to metformin provided no more benefit, overall, than metformin therapy alone. This suggests that type 2 diabetes may progress more rapidly in young people than it does in adults, and that it may be best to start with a more aggressive drug treatment approach in youth with type 2 diabetes. The combination of metformin and rosiglitazone was more effective in treating young people with recent-onset type 2 diabetes than metformin alone. Importantly, however, after the study began, the FDA restricted use of rosiglitazone because of studies linking the medicine to a higher risk of heart attacks and stroke in adults.

**Lifestyle Changes Are a Touchdown for Jameel**

Jameel received the treatment of metformin plus an intensive lifestyle program. The TODAY lifestyle intervention was a family-based weight-management program that included intensive education and

# PATIENT PROFILE

activities delivered one-on-one by trained study staff. While the overall study results didn't show any additional benefit from the lifestyle intervention, Jameel has personally benefitted from taking part in the lifestyle program. "I had to meet with my TODAY coach every couple of weeks. She taught me how to stay healthy by teaching me how to identify and separate good foods from foods that are bad for me, how to manage the size of my meals, how to hydrate myself and cut soda out of my diet, and how to exercise," explains Jameel.

At the time of his enrollment in the TODAY study, Jameel was in middle school and dealing with being overweight. "It was a big deal telling my friends that I had type 2 diabetes. I was uncomfortable, at first, but the coaching helped me to get over my discomfort. The coaching also motivated me, as well as allowed me to talk about things that were personal to me as an adolescent and young adult."

Seven years later, Jameel is still active in the TODAY study. He is now 6 feet tall and weighs 240 pounds, "mainly muscle mass," he says, because of all his exercising and the free weights he continues to use. He adds with a smile, "many of my classmates think I am a member of the university's football team." Jameel continues to see his TODAY coach about every 3 months. At each meeting, his hemoglobin A1c (an indicator of a person's blood sugar levels over the previous 2 to 3 months), height, weight, and blood pressure are measured. He also provides TODAY researchers with an overview of his exercise workout and eating habits. He is still taking metformin.

*"Taking part in the TODAY study has been a great experience," says Jameel. "My coach motivated me and made me want to stay healthy. I would greatly encourage other young people to take part in a study like this."*

Jameel reports, "I feel great. The TODAY study taught me how to eat and exercise properly and how to maintain my diabetes from a young age. As a result, I'm proud to say I've never been hospitalized nor had any complications as a result of my type 2 diabetes."

"Taking part in the TODAY study has been a great experience," says Jameel. "My TODAY coach motivated me and made me want to stay healthy. I would greatly encourage other young people to take part in a study like this."

Staying healthy with what he has learned in the TODAY study and motivated by his education and career ambitions, Jameel's future looks very bright.

For more information about the TODAY study, please see the related advance in this chapter.

# PATIENT PROFILE

## Rachel Bunn

*Battling Cystic Fibrosis-related Diabetes by Participating in a Clinical Trial*

Rachel Bunn with her husband Josh

Rachel Bunn was born 2 months premature, and diagnosed with cystic fibrosis (CF) at the time of her birth. CF is a genetic disorder that results in thick mucus and abnormal sweat, which leads to frequent, serious lung infections, as well as severe complications of the pancreas, liver, digestive tract, and other organs. In years past, children diagnosed with the disease had a predicted median survival of about 10 years, with few surviving beyond their teens. The good news is, due to new treatments made possible by medical research, many people with CF now survive to enjoy many fulfilling years of adulthood.

Unfortunately, as they age, an increasing number of people with CF are developing cystic fibrosis-related diabetes, or CFRD, a form of diabetes that develops as a result of damage to the insulin-producing cells of the pancreas. Rachel learned she had CFRD in her 20s. However, her participation in an NIDDK-supported clinical trial on CFRD has helped determine how she and others with CFRD can control the disease and live longer, healthier lives.

**Living with Cystic Fibrosis**

Rachel has been beating the odds her whole life. She required emergency surgery in her first few days of life to remove an intestinal blockage, a common complication in newborns with CF. Her weight fell to just three pounds, and her health care providers were not optimistic. "At the time, they said I wouldn't survive," says Rachel.

But she did survive, and has continued to battle CF and its complications, including the intestinal obstructions and lung infections, ever since. While normal mucus is watery thin, keeping the lungs healthy and allowing for efficient movement of air, CF causes mucus to be very thick and sticky, increasing risk of serious infection and impeding air flow. As Rachel puts it, "the easiest way to describe what it's like to live with cystic fibrosis is that my lungs are like glue inside instead of like water. When I get infections, the mucus secretions are so thick I can't fight them off like other people do." Instead, Rachel is prescribed powerful antibiotics. If oral medicines don't work, the antibiotics are administered intravenously through PICC lines (peripherally inserted central catheters) to fight off the infection. Rachel says she averages one or two PICC line treatments a year, which last for 2 to 3 weeks at a time. "They're not fun," she says.

# PATIENT PROFILE

*"The easiest way to describe what it's like to live with cystic fibrosis is that my lungs are like glue inside," says Rachel. "When I get infections, the mucus secretions are so thick I can't fight them off like other people do… Sometimes it feels like there's a rubber band around my lungs, and breathing gets difficult."*

In addition to the infections, Rachel has suffered numerous intestinal blockages. One particularly bad blockage occurred 3 months after she was married. "My intestine wrapped around itself, and I was in intense pain," says Rachel. She was rushed to the hospital and underwent 8 hours of emergency surgery, during which surgeons removed more of her intestines.

To maintain her health as best she can, Rachel does a daily regimen of lung therapy, which includes the use of a percussion vest. "The vest helps break up the excess mucus in my lungs," reducing risk of infections, explains Rachel. She also says that diet and exercise help to keep her lungs clear, so she tries to run or swim daily, which is challenging. "I often get tired real easily," she says, "Sometimes it feels like there's a rubber band around my lungs, and breathing gets difficult."

**About CFRD**

In addition to the damaging effects of CF on the lungs, CF interferes with pancreatic function in two important ways, both of which can have the effect of causing serious weight loss. From infancy, the thick mucus of CF patients blocks secretion of pancreatic digestive enzymes needed for absorption of nutrients by the digestive tract and for normal growth. Today, fortunately, babies are screened for CF at birth, and can obtain an adequate supply of these enzymes by taking them in medication form with their meals. Early intervention allows most people with CF to achieve near normal growth.

Indeed, thanks to this and many other improvements in treatment, more and more people with CF are reaching adulthood, which is when the second major pancreatic complication often arises: for reasons that remain unclear, CF can lead to a decline in the capacity of the pancreas to supply the body with insulin, the hormone needed to transport glucose (sugar) into cells. At the same time, also for uncertain reasons, the rest of the body may become less sensitive to insulin. The net result, CFRD, leads to loss of body weight and muscle mass in people who are underweight to begin with. In addition, CFRD increases the decline in lung function, and reduces survival. More research is needed to understand how CFRD affects the function of other organs involved in CF.

Importantly, although CFRD has features in common with type 1 and type 2 diabetes, it is distinct from both. Unlike type 1 diabetes, the insufficient insulin production in CFRD stems not from an autoimmune attack on the pancreas, but rather from a progressive loss of pancreatic function similar to what is seen in type 2 diabetes. And while CFRD involves insulin resistance and has other metabolic and genetic similarities to type 2 diabetes, it is not associated with being overweight or obese.

Indeed, when CFRD was first recognized, clinicians were concerned about its tendency to induce weight loss in CF patients, who are often underweight already. However, it was unclear whether people with the disease were likely to face the same array of other serious complications endured by people with more common forms of diabetes, and which are a major reason for early, aggressive therapy to lower blood sugar in type 1 and type 2 diabetes. Many health care providers

# PATIENT PROFILE

were therefore reluctant to prescribe insulin for CFRD, because no one knew whether insulin, or indeed any drug used to treat other forms of diabetes, would help people with CFRD to be healthier. Furthermore, its use is an additional burden on the patient, and an accidental overdose could cause dangerous hypoglycemia.

**Research Improves Treatment for CFRD**

To provide answers to these important questions and ascertain the benefits and risks of different CFRD treatment options, the NIDDK supported a clinical trial called the Cystic Fibrosis-Related Diabetes Therapy Study (CFRDT Study). Study participants were randomly assigned to one of three groups: 1) to inject pre-meal insulin; 2) to take an oral medication called repaglinide, which is approved for treatment of type 2 diabetes and acts by stimulating the pancreas to secrete more insulin; or 3) to take an oral placebo (an inactive pill given for comparison against the real medication being tested). Participants taking a pill did not know whether they were taking repaglinide or placebo.

Rachel was actually diagnosed with CFRD as a result of being screened for the study, and she was understandably worried. The daily regimens required to control either CF or diabetes are onerous on their own. The prospect of managing both was truly daunting. But she enrolled, recognizing the importance of the study, and was assigned to one of the study arms that received pills. Thanks to her and the other participants, the CFRDT Study was able to demonstrate conclusively that insulin therapy indeed can help people with CFRD maintain their body weight, improve lung function, and feel healthier.

After the results of the study were known, Rachel began to take insulin, which she now administers to herself via an insulin pump. When she was first approached about using a pump, "I thought they were only for people with severe diabetes," she recalls. But, she is happy to report that the insulin pump "changed my life dramatically." While she still has the lung infections and digestive issues associated with CF, her CF-related diabetes has improved. "Thanks to the insulin pump my diabetes is well under control," she explains, and she's on the least amount of insulin that can be administered through the pump.

*Based on the results of the study, Rachel began to take insulin, which she now administers to herself via an insulin pump. She is happy to report that the insulin pump "changed my life dramatically." Today, Rachel says, "I look and feel the best I've ever felt."*

Insulin treatment has also improved her body weight. Prior to entering the study, her body mass index (BMI), a measure of weight in relation to height, was below the normal range. After starting insulin therapy, however, her BMI has gradually risen into the low 20s (normal BMI is between 20-25), which is to say, a very healthy weight. Today, Rachel says, "I look and feel the best I've ever felt."

**Living Life to the Fullest**

Not one to feel sorry for herself because of her CF, or her CFRD, Rachel decided instead to take charge of her life. Now 30 years old, she's been married for 7 years to her husband Josh, an airline pilot; is self-employed as an independent agent for a major insurance company; and enjoys the love and support of family. "I feel extremely fortunate," says Rachel.

Rachel is eager to share her story about benefitting from research, and has done so at different events, including at her local chapter of the Cystic Fibrosis Foundation. "I feel the need to speak up because there just aren't enough

# PATIENT PROFILE

CF patients my age who can do it. Many are on oxygen or have had lung transplants." There's no doubt that Rachel has reacted to her health challenges positively, courageously, and with a desire to help others. When people ask her how she deals with these challenges, Rachel's response is: "10 percent of life is what happens to you; 90 percent is how you react to it."

*Rachel is eager to share her story about benefitting from research. "I feel the need to speak up because there just aren't enough cystic fibrosis patients my age who can do it."*

**Hope Through Research**

The NIDDK is bolstering research on CFRD, to understand its causes and consequences. For example, research will address why some people with CF develop CFRD and others do not, and also examine the molecular mechanisms by which CFRD contributes to a steeper decline in lung function compared to CF patients without diabetes. This knowledge could pave the way toward new prevention and treatment strategies. The NIDDK also continues to support research on CF, including research to develop new therapies, to help Rachel and others with CF live longer, healthier lives, and reduce the burden of managing the disease.


**Each year, the NIH Obesity Research Task Force, consisting of members from many Institutes, Centers, and Offices across the NIH, sponsors seminars held on the main campus in Bethesda, Maryland. These seminars highlight cutting-edge science spanning a broad range of obesity research topics. The posters above advertised the three seminars that were held in 2012.**

# Obesity

*Obesity has risen to epidemic levels in the United States. Individuals who are obese may suffer devastating health problems, face reduced life expectancy, and experience stigma and discrimination. Obesity is a strong risk factor for type 2 diabetes, fatty liver disease, and many other diseases and disorders within the NIDDK's mission.*

*Approximately one-third of U.S. adults are considered obese based on body mass index (BMI), a measure of weight relative to height.[1,2] Nearly 17 percent of children and teens ages 2 through 19 are also obese, and thus at increased risk for developing serious diseases both during their youth and later in adulthood.[3] Obesity disproportionately affects people from certain racial and ethnic groups and those who are socio-economically disadvantaged.*

The high prevalence of obesity in the United States is thought to result from the interaction of genetic susceptibility with behaviors and factors in the environment that promote increased caloric intake and sedentary lifestyles. Research is providing the foundation for actions to address this major public health problem by illuminating the causes and consequences of obesity, evaluating potential prevention and treatment strategies, and providing an evidence base to inform policy decisions. The NIDDK supports a multi-dimensional research portfolio on obesity, spanning basic, clinical, and translational research. NIDDK-funded studies investigate a variety of approaches for preventing and treating obesity. These span behavioral and environmental approaches in families, schools, and other community settings; medical and surgical interventions; and combinations of these strategies. In parallel, Institute-supported investigations into the biologic processes associated with body weight may spark new ideas for intervention approaches. To help bring research results to health care providers and the public, the Institute also sponsors education and information programs.

The NIDDK also continues to play a leading role in the NIH Obesity Research Task Force. The NIDDK Director co-chairs the Task Force along with the Directors of the National Heart, Lung, and Blood Institute and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development. The Task Force includes representatives from these and numerous other NIH Institutes, Centers, and Offices. In 2012, members of the Task Force collaborated with other major research and health organizations and HBO to develop *The Weight of the Nation*, a documentary series and public education initiative that spotlights this urgent public health problem. NIDDK staff and grantees, as well as staff from other NIH Institutes, provided extensive scientific guidance for *The Weight of the Nation* films.

Highlights of recent advances from NIDDK-supported research on obesity are provided in this chapter. These represent examples of NIDDK's broad spectrum of research efforts toward reducing the burden of obesity so that people can look forward to healthier lives.

---

[1] *Statistics Related to Overweight and Obesity. http://win.niddk.nih.gov/statistics/index.htm*

[2] *Flegal KM, et al: JAMA 307: 491-497, 2012.*

[3] *Ogden CL, et al: JAMA 307: 483-490, 2012. For children and adolescents, obesity refers to a BMI at or greater than the 95th percentile on growth charts (which are based on previous national surveys).*

## FOOD ON THE BRAIN

**Glucose Levels Affect Desire for High-calorie Food:** Scientists have found that blood glucose levels can stimulate or restrain interest in high-calorie food, but that this regulatory mechanism may be lost in the context of obesity. Normally, the body strives to keep blood levels of glucose, the main cellular energy source, within a tight range. In healthy individuals, a transient drop in blood glucose levels, or hypoglycemia, stimulates hunger and food seeking behavior, whereas eating a meal restores blood glucose levels to a normal range ("normoglycemia") and satiates hunger. However, not just any food will do—during mild hypoglycemia, people preferentially seek out foods high in sugar and fat. The brain is central to many mechanisms driving feeding behavior, but the precise brain pathways driving the motivation to consume high-calorie foods have not been known, nor whether there are differences between obese and non-obese individuals.

In the current study, researchers investigated whether mild hypoglycemia stimulates activity in parts of the brain linked to motivation and reward and if that activity is associated with desire for calorie rich foods. Using an imaging technology called functional magnetic resonance imaging (fMRI), the researchers examined changes in brain activity in obese and non-obese people as they were shown pictures of food. Participants viewed images of high-calorie foods (*e.g.*, ice cream), low-calorie foods (*e.g.*, carrots), and non-foods first while in a normoglycemic state, then while in a mildly hypoglycemic state. As they viewed each image, participants were also asked to rate how much they liked and wanted the item they saw. When compared to their reactions during normoglycemia, people experiencing hypoglycemia showed greater activity in motivation and reward regions of the brain when viewing high-calorie foods, and their desire for them increased. In contrast, low-calorie food images did not provoke the same changes in brain activity and desire during hypoglycemia. Moreover, in non-obese participants, normoglycemia was associated with greater activity in brain regions that reduce motivation for rewarding stimuli—a response that was generally associated with lower desire for any of the foods. However, in obese individuals, this repressive brain activity was lost.

These findings not only help identify brain regions involved in food motivation, but also suggest that higher or lower blood glucose levels can influence susceptibility to high-calorie food cues in the environment—and that persons who are obese may be more susceptible, due to a loss of this regulatory response. While further research is needed, these findings also suggest that it may be possible to develop strategies to reduce desire for high-calorie food by minimizing hypoglycemia between meals.

*Page KA, Seo D, Belfort-DeAguiar R, et al. Circulating glucose levels modulate neural control of desire for high-calorie foods in humans. J Clin Invest 121: 4161-4169, 2011.*

**Discovery of Factor in the Brain That Regulates Appetite:** Scientists have identified a factor in the brain, called Gpr17, that has a central role in regulation of appetite in mice. It is known that damage to the hypothalamus—a part of the brain that functions to connect the nervous system to the endocrine system—leads to changes in hunger, satiety, and physical activity. Hypothalamic neurons (nerve cells) that produce a protein called AgRP have been directly implicated in promoting feeding behavior: studies have demonstrated that activation of AgRP neurons rapidly increases food intake, while deletion of AgRP neurons causes cessation of feeding and results in starvation.

The hormones insulin and leptin have been previously shown to inhibit the activity of AgRP neurons, but disruption of insulin or leptin signaling specifically within these nerve cells has mild to no effect on feeding behavior, indicating that neither pathway has sole control over how these cells contribute to appetite regulation. In addition, obesity can lead to insulin and leptin resistance. Scientists sought to identify additional pathways in AgRP neuron-dependent food intake that could potentially be targeted by drug therapy to inhibit the activity of AgRP neurons.

A protein called FoxO1 integrates both leptin and insulin signaling, so the researchers generated genetically engineered mice that lack FoxO1 in AgRP neurons and looked to see if removing this protein affected the appetite of the mice. They found that mice lacking FoxO1 in their AgRP neurons are lean, eat less, and show improved glucose control, as well as increased sensitivity to insulin and leptin. Because FoxO1 is a poor drug target, the scientists sought to determine another target with better therapeutic prospects. To do so, they looked for genes whose activity was reduced in FoxO1-deficient AgRP neurons. They identified the gene encoding Gpr17 as a prominent FoxO1 target and found that inhibition of Gpr17, by injecting a chemical into mice, reduced food intake and increased brain sensitivity to hormones and nutrients.

Gpr17 is also found in humans and is part of a family of proteins that is considered "highly druggable"—a number of existing drugs work through this family. In addition, Gpr17 is abundant in AgRP neurons but not in other neurons, potentially minimizing unwanted drug side effects. Additional research will be necessary to demonstrate whether inhibition of Gpr17 leads to the same outcomes in humans, but these findings reveal a new signaling pathway with potential targets to control appetite and obesity.

*Ren H, Orozco IJ, Su Y, et al. FoxO1 target Gpr17 activates AgRP neurons to regulate food intake. Cell 149: 1314-1326, 2012.*

**Brain Injury Associated with High-fat Diet and Obesity:** In people who are obese, and in rodents fed a high-fat diet, researchers discovered damage to an area of the brain that regulates body weight. Previously, scientists had observed inflammation in the brain of rodents with diet-induced obesity. In the current study, a team of researchers further investigated this adverse process. They began with rats and mice that were particularly genetically susceptible to obesity from a high-fat diet. Within a day on a high-fat diet, the rodents' brains began to react as if they had suffered serious injury. Genes that promote inflammation were activated, and immune cells called microglia hastened to an area of the brain, the hypothalamus, that controls appetite and body weight. Within 3 days, these microglial cells had increased both in number and size. Within a week, astrocytes—another type of brain cell—had responded as well; the normally discrete projections that branch out from these cells had wrapped into a dense mass. The researchers also observed induction of a protein, Hsp72, known to help protect cells from injury. Although set in motion by a high-fat diet in the present study, these types of brain changes have also been seen in response to brain damage resulting from disruption of blood flow to the brain and even Parkinson's and Alzheimer's diseases. Some of the inflammatory and cellular changes were transient at first, as though the brain were attempting to limit adverse effects of the diet, but then reappeared as the high-fat feeding continued; other changes persisted unabated throughout the months of unhealthy eating. Many of the changes began rapidly, even before the animals gained substantial body weight. The researchers then found evidence of brain cell death—specifically of brain cells called POMC neurons, which normally reduce appetite and have other functions that help prevent obesity. With fewer POMC neurons, the likelihood of obesity increases. To see whether similar brain changes occur in people, the researchers analyzed MRI images that had been taken previously. Close inspection of brain images from 34 people revealed differences between lean and obese individuals, with evidence of changes in the hypothalamus of the brain. Thus, this study suggests that obesity and high-fat diet consumption are associated with damage to the brain.

*Thaler JP, Yi CX, Schur EA, et al. Obesity is associated with hypothalamic injury in rodents and humans. J Clin Invest 122: 153-162, 2012.*

## NEW DISCOVERIES ABOUT BROWN FAT

**Newly Identified Muscle Hormone May Have Potential for Reducing Obesity and Type 2 Diabetes:** Recent research shows that in both mice and humans, exercise induces muscle to release a newly discovered hormone, irisin, and studies in mice show that irisin promotes energy expenditure (calorie burning), and

reduces obesity and type 2 diabetes. The mammalian body contains two kinds of adipose (fat) tissue: white adipose tissue (WAT), which stores fat for energy, and brown adipose tissue (BAT), which "burns" fat to help maintain body heat without shivering—thereby increasing the body's energy expenditure. Although human brown fat was initially thought to be present only in newborns, recent studies have confirmed its presence and function in adults. A new study has identified a hormone, called irisin, which is produced by muscle tissue and instructs WAT to take on BAT-like characteristics. When irisin was administered to adult mice or added to mouse WAT cells, genes normally found in BAT were turned on, whereas some WAT genes were turned off. The researchers found that in mice and in human study participants, exercise led to an elevation in circulating irisin levels. When the scientists modestly increased the amounts of circulating irisin in a mouse model of type 2 diabetes, this treatment reduced obesity and improved blood glucose control without apparent side effects. These results reveal a hormone that appears to drive many of the physiological benefits of exercise. If irisin in humans works as it does in mice, administration of this hormone could be a potential new therapeutic approach for obesity and type 2 diabetes.

*Boström P, Wu J, Jedrychowski MP, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 481: 463-468, 2012.*

**New Insight into Adult Human Brown Fat Identity:** Scientists have shown that adult human brown fat may actually be "beige" fat—a distinct tissue that burns energy and could serve as a potential target for novel therapies for obesity. The human body has been thought to possess two kinds of fat cells: white fat cells, which store fat molecules; and brown fat cells, found most abundantly in infants, which burn calories and generate heat. However, in mice, for many years scientists have observed, embedded in white fat, a third type of fat cell—now called "beige" fat—that shares characteristics of both brown and white fat. While it was clear that beige fat cells resemble brown fat in appearance and function, little was known about their properties, development, and activity. In a recent study, researchers developed methods to isolate and characterize beige fat cells from certain areas of mouse white fat tissue (subcutaneous white fat), and found that they exhibit unique properties, as well as some characteristics of classical brown fat cells. By isolating these cells, the scientists could then carefully define the set of genes that were turned on specifically in beige fat. The scientists also isolated cells that have the potential to become beige fat—cells called "precursors." They showed that these beige fat precursors were responsive to the hormone irisin, which is known to convert white fat tissue to a more brown fat-like identity. The researchers then utilized their new knowledge from mice to better characterize adult human brown fat deposits, and found that the fat cells within more closely resembled beige fat than they did classical brown fat cells. This greater understanding of beige cell properties may lead to the development of potential therapies for obesity through the activation of energy-burning adult human beige fat tissue.

*Wu J, Boström P, Sparks LM, et al. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 150: 366-376, 2012.*

**Ephedrine Does Not Activate Brown Fat:** A recent study has found that in humans, brown fat tissue is activated by mild cold temperature exposure, but not by the drug ephedrine, a decongestant and bronchodilator known to induce weight loss. Many scientists believe that brown adipose tissue (BAT)—a type of fat tissue known to increase energy expenditure, which in turn can promote weight loss—could serve as an ideal target for the development of treatment strategies for obesity. While mild cold temperature exposure is known to activate BAT, safe and effective pharmacological agents could offer a more practical therapeutic approach. Research scientists sought to determine whether ephedrine could induce BAT activation. Ephedrine is known to stimulate the sympathetic nervous system—the portion of the nervous system broadly responsible for the "flight or fight" response and also for the stimulation of BAT. Healthy adult volunteers were either given ephedrine injections, a "cooling vest" set to 57 degrees Fahrenheit, or saline injections as a control. The scientists found that cold

exposure, but not ephedrine treatment, activated BAT tissue. In both ephedrine and cold exposure treatment conditions, energy expenditure, basal metabolic rates, and blood pressure increased. However, heart rates were raised with ephedrine treatment, but reduced with cold exposure. These results demonstrated that ephedrine does not induce weight loss through BAT activation. Further research aimed at understanding the brain and molecular pathways that activate BAT upon exposure to cold temperatures may reveal new avenues for the development of anti-obesity therapeutics.

*Cypess AM, Chen Y-C, Sze C, et al. Cold but not sympathomimetics activates human brown adipose tissue in vivo. Proc Natl Acad Sci USA 109:10001-10005, 2012.*

**The Quest To Make Visceral Fat Burn Itself:** Seeking to learn what sparks the appearance of calorie-burning "brown fat" cells within the body's visceral fat tissue, researchers have identified their origins, or progenitor cells, in mice, and molecular regulators in both mouse and human tissue that may lead to new therapeutic strategies for obesity and related diseases. Humans and mice harbor different types of fat tissue. Calorie-storing "white fat" tissue is the most abundant, and can be found surrounding internal organs, where it is referred to as visceral or abdominal fat, and just under the skin, a location termed subcutaneous. Brown fat, which burns calories to generate heat, also takes various forms, but is more transient. Babies and rodents have brown fat tissue that, in the case of humans, largely disappears after infancy. However, cells with characteristics of brown fat (sometimes called beige or brite cells) can also appear within patches of white fat tissue, in response to cold or other nervous-system triggers. Typical brown fat tissue develops from progenitor cells that are related to another calorie-burning tissue, muscle, but the origin of brown fat cells that arise within white fat tissue has been unknown. Based on previous reports that suggested a protein called PDGFRα might mark such progenitor cells, scientists tagged visceral fat cells in mice in a way that would mark not only progenitor cells with this protein, but also all cells descending from them. Subsequently, they put some of the mice on a high-fat diet for 8 weeks, and gave the other mice a chemical that stimulates factors in the nervous system (β3-adrenergic receptors) to cause heat generation. Examining the tagged cells, the researchers found that after β3-adrenergic stimulation, the progenitors gave rise to new cells with characteristics of brown fat. By contrast, the high-fat diet led to new white fat cells. Having illuminated two divergent paths for visceral fat progenitor cells, scientists may develop therapies that steer these cells toward brown fat development.

In other recent studies, scientists explored ways to remove molecular barriers to brown fat development within visceral white fat tissue. One team focused on a protein called ActRIIB, which limits muscle mass and regulates fat tissue. Building on previous research, they developed a "decoy" version, ActRIIB-Fc, to subvert the effects of the normal protein, and administered it to mice along with a high-fat diet. Compared with mice that did not receive the decoy, those that did had increased lean tissue (muscle) mass and less fat tissue; they were protected from metabolic effects of a high-fat diet, such as abnormal fat accumulation in the liver; and within their visceral fat were cells that had activated genes characteristic of brown fat. While this study shows that ActRIIB-Fc can prevent diet-induced obesity, future research may determine whether it could help treat mice that are already obese, and whether this approach may work in people. Pursuing an alternate route from white to brown fat, another research team investigated a molecule called retinaldehyde, which is related to vitamin A and is found in white fat, along with an enzyme that processes it, called Aldh1a1. The researchers observed the enzyme in visceral white fat, with higher levels observed in mice fed a high-fat diet and in people who were extremely obese. A chemical inhibitor of this enzyme, when injected into obese mice, limited further weight gain from a high-fat diet; improved their glucose levels, a sign of reduced diabetes risk; and activated brown fat genes in visceral fat tissue.

By revealing previously unknown brown fat progenitor cells and exploring factors that regulate brown fat development, these and other studies may lead to new obesity therapies that coax cells in visceral white fat tissue to burn calories like brown fat.

*Koncarevic A, Kajimura S, Cornwall-Brady M, et al. A novel therapeutic approach to treating obesity through modulation of TGFβ signaling. Endocrinology 153: 3133-3146, 2012.*

*Kiefer FW, Vernochet C, O'Brien P, et al. Retinaldehyde dehydrogenase 1 regulates a thermogenic program in white adipose tissue. Nat Med 18: 918-925, 2012.*

*Lee YH, Petkova AP, Mottillo EP, and Granneman JG. In vivo identification of bipotential adipocyte progenitors recruited by β3-adrenoceptor activation and high-fat feeding. Cell Metab 15: 480-491, 2012.*

**Identification of a Protein Controlling Energy Expenditure and Inflammation in Fat Tissue:** Researchers have identified a protein in adipose (fat) tissue of mice that regulates both energy expenditure (calorie burning) and inflammation, making it a promising target for treating obesity and type 2 diabetes. Mammals have two major types of fat tissue: brown adipose tissue (BAT) burns fat and thereby increases energy expenditure, and white adipose tissue (WAT), the more abundant form, stores fat. A promising approach to treating obesity and related diseases is to make WAT take on BAT-like characteristics to increase whole-body energy expenditure. Toward this goal, researchers sought to identify factors that regulate energy expenditure. They identified an ion channel protein, TRPV4, that is found in high levels in WAT. In cells grown in laboratory culture, experimentally blocking TRPV4 made WAT become more like BAT by activating genes that increased energy expenditure. Blocking TRPV4 in fat cells was also found to have an unexpected benefit—it decreased the activity of genes important in promoting inflammation. Obesity is associated with chronic, low-grade inflammation of fat tissue, which contributes to the development of insulin resistance, a condition associated with type 2 diabetes; therefore, identifying ways to reduce inflammation is an important goal. These results suggest that TRPV4 regulates both energy expenditure and inflammation in fat tissue, even though scientists previously thought that the molecular mechanisms regulating those processes were distinct. To see if TRPV4 played this dual role in animals, the researchers conducted experiments using mice that were genetically engineered to lack TRPV4. On a regular diet, the experimental mice weighed the same as control mice. However, when the animals were fed a high-fat diet for 16 weeks, the mice lacking TRPV4 not only gained less fat weight than control mice, but were also protected from fat tissue inflammation and insulin resistance. They also had increased energy expenditure without differences in food intake, physical activity, or body temperature compared to control mice, suggesting that the increased energy expenditure was due, at least in part, to increased fat burning. Because these findings suggest that TRPV4 may be a promising drug target for treating obesity, the scientists next used, as a potential drug, a chemical that inhibits TRPV4 activity in obese mice. Compared to control mice, the treated animals had improved glucose tolerance, and their fat tissue showed increased activation of energy burning genes and decreased activation of genes involved in inflammation. These results suggest that inhibiting TRPV4 gives a two-fold benefit of increasing energy expenditure and reducing inflammation in fat tissue. If these findings are extended to humans, targeting the protein may be a therapeutic avenue for treating obesity and type 2 diabetes.

*Ye L, Kleiner S, Wu J, et al. TRPV4 is a regulator of adipose oxidative metabolism, inflammation, and energy homeostasis. Cell 151: 96-110, 2012.*

## CELLULAR TARGETS FOR REDUCING OBESITY AND METABOLIC SYNDROME

**Getting Rid of Body Fat by Targeting Its Blood Supply:** Results of recent research suggest it may one day be possible to achieve weight loss by targeting cells in the blood vessels of body fat. In previous work with mice, the researchers used a molecule called "adipotide" that binds only to blood vessels in white adipose tissue, which accounts for the great majority of fat in the body. The molecule triggers cell death specifically in the cells to which it binds. Although the mice given adipotide lost a great deal of weight,

and seemed to suffer no obvious ill effects from the treatment, numerous previous weight loss methods that seemed promising in mice have failed to prove effective in humans. In the new research, therefore, scientists tested the approach in obese monkeys—an animal model that more closely mimics human obesity. Just as with humans, some monkeys become overweight or obese when given an abundant supply of whatever food they want to eat, while others do not. Also, as with humans, obesity in monkeys can lead to insulin resistance, type 2 diabetes, and other metabolic and cardiovascular problems. The scientists injected obese monkeys with doses of adipotide or placebo daily for 4 weeks, and then followed the animals for an additional 4 weeks. The monkeys receiving adipotide began to lose a significant amount of weight within the first week of treatment, and continued to lose weight for 3 weeks after treatment ended, losing a total of about 15 percent of their body weight in 7 weeks. Tests of body composition revealed that almost all of the weight lost was body fat, although mild dehydration did also occur. (In a separate experiment, the researchers showed that lean monkeys treated with adipotide did not lose weight, indicating that the drug works selectively on fat tissue.) The obese animals receiving adipotide ate less than their counterparts, although they seemed to continue to like their food. In other respects, their behavior was normal, during and after treatment. Adipotide-treated monkeys produced more urine than did control animals, and had significant but not serious increases in urine glucose and protein levels that reverted to normal after treatment. Notably, adipotide treatment also improved insulin sensitivity. Of some concern, the treatment also led to an elevation of serum creatinine, a sign of kidney damage. The elevated creatinine levels seen in the adipotide-treated monkeys fell during recovery, but remained slightly higher than in control animals. Direct examination of the kidneys revealed signs of slight damage which appeared to be reversible, but which suggests a possible side effect to watch for in potential future human trials. These results validate the general approach of reducing body fat by attacking its blood supply. Future research will determine whether adipotide or a similar agent would be safe and effective for weight loss in humans.

*Barnhart KF, Christianson DR, Hanley PW, et al. A peptidomimetic targeting white fat causes weight loss and improved insulin resistance in obese monkeys.* Sci Transl Med *3: 108ra112, 2011.*

**Working Out the Health Benefits of Autophagy:**
Researchers have discovered that an intracellular protein degradation system may be crucial to normal metabolism and the response to exercise. To help get rid of imperfect proteins and aging cellular components, and in some cases to modulate levels of intracellular factors, a cell can engulf portions of itself, target the damaged contents for breakdown, and recycle undamaged components for reuse. This process is called autophagy. While autophagy occurs at a basal level under normal circumstances, it is also stimulated by starvation and other stressors, enabling cells to adapt to changing conditions and needs. Scientists studying this process in mice recently found that muscle cells turn up autophagy in response to exercise. Intriguingly, when mice with mutations that hinder such stimulus-induced autophagy ran on a treadmill without prior exercise training, they showed lower endurance than normal mice. Also, while strenuous exercise normally induces changes in skeletal muscle that help it use glucose more efficiently, the autophagy-deficient mice did not show those changes. Autophagy was particularly important for achieving the metabolic benefit of long-term exercise training, in the context of obesity. Researchers found that while exercise protected normal mice from elevated glucose levels induced by a high-fat diet, it did not give autophagy-deficient mice the same protection. The autophagy-deficient mice also did not show exercise-induced improvements in levels of cholesterol and triglycerides (a type of fat) that were seen in normal mice. These findings suggest that autophagy plays a role in conferring the health benefits of exercise.

In addition to evidence that autophagy helps the body respond to exercise, researchers found it is also needed to produce a signal that the body has consumed enough food. Scientists studied the role of autophagy in the hypothalamus, the region of the brain central to control of energy balance—regulating energy intake (as food calories) and expenditure (burning calories to maintain basic body functions, do work, or generate heat).

Certain cells in the hypothalamus generate the hormone α-melanocyte-stimulating hormone (α-MSH) that signals the brain to curtail eating and promote calorie burning. Mice genetically engineered to lack autophagy in just these brain cells showed altered levels of molecular factors important to energy balance, including reduced levels of α-MSH. These mice gained more fat weight than normal mice when fed a high-fat diet, and appeared to have problems with mobilizing fat from fat cells for use as an energy source when fasted. They also showed impaired glucose tolerance. Similar problems with brain-derived hormone levels and with fat mobilization occur with older age in mice—raising the possibility that some of the metabolic problems associated with aging may be due partly to loss of autophagy.

Together, these papers suggest that autophagy, already known to help protect against cancer and some other diseases, also has a key role in counteracting some of the dangerous metabolic consequences of obesity, including type 2 diabetes risk. If borne out through further research, strategies to mimic or manipulate autophagy may prove beneficial in preventing or treating these conditions.

*He C, Bassik MC, Moresi V, et al. Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis. Nature 481: 511-515, 2012.*

*Kaushik S, Arias E, Kwon H, et al. Loss of autophagy in hypothalamic POMC neurons impairs lipolysis. EMBO Rep 13: 258-265, 2012.*

**Diet-induced Changes in Fat Tissue—Role of the *FGF1* Gene and Link to Type 2 Diabetes:** Scientists discovered that the *FGF1* gene plays a key role in "remodelling" body fat tissue in rodents in response to dietary changes. These findings may ultimately lead to new treatments for type 2 diabetes and for reducing the elevated glucose levels often found in obese individuals. Body fat tissue typically changes to accommodate the influx of nutrients encountered following re-feeding after an overnight fast, or after a switch from a normal to a high-fat diet. In experiments to understand how these metabolic changes occur, researchers discovered that the *FGF1* gene was activated in the fat tissue of mice that ate normal chow compared to those that had fasted overnight, and *FGF1* induction increased even more when the mice were given a high-fat diet. The *FGF1* gene was turned on by the master regulator of fat tissue, a protein called PPARγ. To gain further insights, the research team compared normal mice to those genetically engineered to lack *FGF1*. When allowed to eat as much as they wanted of a high-fat diet, mice lacking *FGF1* gained the same amount of weight as normal mice, but otherwise their health was far worse. They developed severe type 2 diabetes; their livers were enlarged and contained excess fat; and, potentially at the root of these problems, their visceral fat tissue was abnormal. Although excess nutrients usually cause fat tissue to expand, the visceral fat of *FGF1*-deficient mice did not; it instead showed structural defects and more inflammation than that of normal mice. Conversely, when switching mice from a high-fat diet to healthier fare, the researchers also found that *FGF1* helped fat tissue adapt accordingly. These new findings suggest that *FGF1* helped mitigate such problems by directing fat tissue to adjust to feeding, fasting, and—at least to some extent—a high-fat diet. Although *FGF1* had been implicated in other biological processes, its crucial functions in fat tissue were initially surprising to the researchers because in previous studies, mice without *FGF1* seemed perfectly fine. However, until this new study, scientists had not examined *FGF1*-deficient mice on a high-fat diet. The new study suggests that in mice, as in people, genetic susceptibility to type 2 diabetes and other metabolic problems may only become apparent when on an unhealthy diet, particularly one that leads to obesity. These new findings point to *FGF1* as a potential target for developing novel type 2 diabetes therapies.

*Jonker JW, Suh JM, Atkins AR, et al. A PPARγ-FGF1 axis is required for adaptive adipose remodelling and metabolic homeostasis. Nature 485: 391-394, 2012.*

## RESEARCH ON THE EFFECTS OF BARIATRIC SURGERY

**Bariatric Surgery Reduces Blood Glucose Levels:** A recent study has shown that bariatric surgery can help control type 2 diabetes more

effectively than medical therapy alone, and can help reduce the need for medications to lower glucose, lipids, and blood pressure. To understand the potential health benefits of bariatric surgery for people with obesity and poorly controlled type 2 diabetes, researchers compared outcomes achieved through intensive medical therapy (which included lifestyle counseling, weight management programs, frequent home glucose monitoring, and the use of diabetes medications) to those obtained with intensive medical therapy in combination with bariatric surgery. Of the many available forms of bariatric surgery, researchers tested two specific procedures: a variation of gastric bypass surgery, called Roux-en-Y surgery, in which the top portion of the stomach is connected directly to a lower portion of the small intestine; and sleeve gastrectomy, in which the majority of the stomach is removed, leaving a comparatively narrow "sleeve." After 12 months, blood glucose was reduced to levels below the diabetic range in only 12 percent of participants that received medical therapy alone, compared to 42 percent in the gastric bypass group and 37 percent in the sleeve gastrectomy group. Indeed, many of the patients in the two surgery groups who achieved these good glucose levels within a year of surgery did so without further use of diabetes medications. Overall, the use of medications to treat cholesterol and blood pressure, as well as to lower blood glucose levels, decreased sharply in both surgical procedure groups, whereas medication use modestly increased in the group that was given medical therapy alone. Longer studies will be needed to determine whether the metabolic improvements observed in the surgery patients will be durable. Further, determining whether these results apply equally to various racial/ethnic groups with obesity and type 2 diabetes will require a larger study, with a more diverse cohort. However, this study adds to existing evidence that bariatric surgery may be a reasonable approach for treating some patients with obesity and uncontrolled type 2 diabetes.

*Schauer PR, Kashyap SR, Wolski K, et al. Bariatric surgery versus intensive medical therapy in obese patients with diabetes. N Engl J Med 366: 1567-1576, 2012.*

**Health Benefits 6 Years After Bariatric Surgery:** While the long-term health risks of extreme obesity have been well documented, a recent study has provided important new information on long-term health benefits of a major form of treatment—bariatric surgery. People who have extreme obesity typically do not gain sufficient health benefits from lifestyle intervention alone or from lifestyle plus drug treatment, and thus many turn to bariatric surgery. Although researchers have reported that bariatric surgery can lead to significant weight loss and improvements in type 2 diabetes and other metabolic conditions over the short term, there has been only limited information on long-term effects. To gain more data on risks and benefits over the longer term, a team of researchers assessed health outcomes 6 years after surgery. For the study, they recruited over 400 individuals who were extremely obese and who had Roux-en-Y gastric bypass surgery, a common type of bariatric surgical procedure. For comparison, the researchers also recruited several hundred individuals who were similarly obese but did not undergo surgery. A majority of the study volunteers were women. The surgery led to a number of health benefits that persisted for years. Individuals who had surgery experienced better weight-loss maintenance, with a majority (76 percent) having kept off 20 percent of their initial body weight for 6 years after surgery. Among participants who had type 2 diabetes at the beginning of the study, those receiving surgery had a 62 percent rate of disease remission 6 years later, compared to only a 6 to 8 percent remission rate seen in those who did not have surgery. Among participants who did not have diabetes at the outset of the study, those who had surgery were less likely to develop the disease. Other outcomes seen after surgery included higher levels of "good" cholesterol (HDL), lower levels of "bad" cholesterol (LDL) and triglycerides, and better blood pressure. Although the overall effects of surgery were beneficial, some of the individuals (approximately 8 percent) required further hospitalization after surgery, and there were four suicides reported. The reasons for the small number of suicides, which was significantly higher than in the control population, are unknown, but this finding indicates a need for greater attention to patients'

psychological health before and after surgery. Taken together, the findings from this study add important long-term data to the current knowledge about bariatric surgery and will help individuals and their health care providers with treatment decisions. Future research may show whether the results are similar in diverse racial/ethnic groups, as most of the participants in this study were non-Hispanic white. Finally, although the participants' health was tracked over 6 years, the entire study took over a decade to complete, demonstrating the value of long-term research efforts.

*Adams TD, Davidson LE, Litwin SE, et al. Health benefits of gastric bypass surgery after 6 years. JAMA 308: 1122-1131, 2012.*

**Weight-loss Surgery Increases Risk for Alcohol Use Disorders Over Time:** A recent study showed that adults who had Roux-en-Y gastric bypass (RYGB) bariatric surgery to lose weight had a significantly higher risk of alcohol use disorders (AUD) 2 years after surgery compared with before surgery. Researchers investigated alcohol consumption and AUD symptoms in 1,945 participants from the Longitudinal Assessment of Bariatric Surgery (LABS), a prospective study of patients undergoing weight-loss surgery at one of 10 different hospitals across the United States. Within 30 days before surgery and again 1 and 2 years after surgery, study participants completed the Alcohol Use Disorders Identification Test (AUDIT), a validated and reliable alcohol use screening method, to identify symptoms of AUD. Study participants were categorized as having AUD if they were positive for at least one symptom of alcohol dependence or alcohol-related harm, or if their total AUDIT score was at least 8 (out of 40). Among participants who had the RYGB procedure, 7.0 percent reported symptoms of AUD prior to surgery. There was no significant increase in AUD 1 year after surgery. However, by the second postoperative year, 10.7 percent of patients reported symptoms of AUD, a relative increase of more than 50 percent compared to pre-surgical rates. Patients who underwent another common type of weight-loss surgery, laparoscopic adjustable gastric banding (LAGB), did not report an increase in symptoms of AUD. About 70 percent of the study participants had RYGB surgery, another 25 percent had LAGB surgery, and about 5 percent of the patients had other, less common weight-loss surgeries. AUD prior to surgery was one of the strongest predictors of postoperative AUD, although more than half of the study participants with AUD after surgery did not report having the condition during the year before surgery. In addition, patients with less social support or who reported preoperative recreational drug use or smoking were more likely to report symptoms of AUD after surgery. Men and younger adults were also more likely to develop AUD. Depressive symptoms, mental health treatment, and binge eating prior to surgery were not independently related to an increased likelihood of AUD after surgery. The study results suggest that clinicians should be aware of the importance of monitoring for signs and symptoms of AUD and consider counseling after bariatric surgery, particularly in patients who undergo gastric bypass.

*King WC, Chen J-Y, Mitchell JE, et al. Prevalence of alcohol use disorders before and after bariatric surgery. JAMA 307: 2516-2525, 2012.*

## INSIGHTS INTO DIET AND ACTIVITY—STRATEGIES AND HEALTH BENEFITS

**Weight Loss and Increased Fitness Slow the Decline of Mobility in Adults:** New research has shown that weight loss and increased physical fitness reduce the risk of losing mobility in overweight or obese adults with type 2 diabetes. Older adults with type 2 diabetes are more likely to have reduced mobility than those without this disease, and obesity increases the risk for mobility-related health problems. As part of the Look AHEAD (Action for Health in Diabetes) clinical trial, researchers investigated whether a lifestyle intervention program could slow the reduction of mobility. Look AHEAD is determining whether a lifestyle intervention designed to promote weight loss can improve health outcomes in overweight or obese people with type 2 diabetes. Participants were randomly assigned to either an intensive lifestyle intervention group (ILI) or a diabetes support and education group (DSE). Among

the tests done in the trial, the researchers measured participants' weight, and they assessed the participants' fitness with a treadmill test. When the Look AHEAD trial began, nearly two-thirds of participants reported mild, moderate, or severe restrictions in mobility. After 4 years of the study, participants in the ILI group did not lose as much mobility as those in the DSE group. The ILI intervention slowed decline in mobility by 48 percent compared to DSE. Moreover, 20.6 percent of ILI participants reported severe disability compared to 26.2 percent of participants in the DSE group. Similarly, 38.5 percent of those in the ILI group reported good mobility, whereas the rate was 31.9 percent in the DSE group. Weight loss was a slightly stronger predictor of better mobility than was improved fitness, but both contributed significantly to the observed reduction in risk. These results are consistent with previous analyses, which showed that participants in the ILI group lost significantly more weight than did those in the DSE group, and also had improved fitness, glucose control, blood pressure, and HDL cholesterol with less use of medication. These findings show that intensive lifestyle intervention programs can slow the decline of mobility in overweight or obese people with type 2 diabetes, and have significant implications for improving quality of life as people age.

*In September 2012, the intervention was discontinued when it was found that intensive lifestyle intervention in overweight/obese adults with long-standing type 2 diabetes did not reduce cardiovascular events such as heart attack and stroke. Although the intervention did not reduce cardiovascular events, Look AHEAD has previously shown other important health benefits of the lifestyle intervention, including decreasing sleep apnea, reducing the need for diabetes medications, improving quality of life, and helping to maintain physical mobility (as shown in this advance). Although the intervention was discontinued, follow-up of all study participants will continue to evaluate their long-term health and effects of the weight loss intervention.*

*Rejeski WJ, Ip EH, Bertoni AG, et al. Lifestyle change and mobility in obese adults with type 2 diabetes. N Engl J Med 366: 1209-1217, 2012.*

**Physical Activity and Reduced Risk for Obesity in Adults with *FTO* Gene Variants:** Although we cannot change our genome sequence, we can influence some of the effects of our genes. Researchers found that in adults, physical activity may reduce the risk for obesity associated with certain variants of the *FTO* gene. Knowing that people can have different forms of the *FTO* gene, which vary slightly in their gene sequences, the researchers compared people who have a form of the *FTO* gene that increases risk for obesity with individuals who have another form of the gene. Because previous reports had conflicting results as to whether physical activity can attenuate this risk, the team of researchers decided to analyze more people. To do this, they compiled information on *FTO* gene sequences and physical activity for over 218,000 adults who had participated in a variety of other studies. For the new analysis, the researchers defined adults as "inactive" if they had sedentary jobs and less than an hour per week of moderate-to-vigorous activity during leisure time or commuting, or if their activity levels were otherwise particularly low compared to other participants of their respective study. Those with higher activity levels were considered "physically active." Overall, they found that people with the adverse *FTO* gene variant were more likely to be obese than those with the other form of the *FTO* gene. Encouragingly, physical activity reduced this risk by 27 percent. In a similar analysis of over 19,000 children, the researchers found that physical activity, which was defined slightly differently for this age group, did not seem to attenuate *FTO*-specific risks for obesity, and it did not correlate with the children's body mass index (a measure of weight relative to height). However, physical activity did appear to be helpful for children in general, as those who were physically active had less body fat and a smaller waist circumference than inactive children. Other studies of the *FTO* gene have found that adverse *FTO* variants seem to drive people to eat more, particularly high-fat foods. It is not clear how, in adults, physical activity may reduce obesity risk conferred by the *FTO* gene, or whether the reduced risk may be due to a combination of lifestyle factors. For example, adults who are physically active might also tend to have healthier eating habits than those who are sedentary. These results offer hope of reducing obesity risk for the

many people with this *FTO* gene variant, and emphasize the value of both individual behaviors and environments that promote healthy lifestyles, regardless of one's genetic predisposition.

*Kilpeläinen TO, Qi L, Brage S, et al. Physical activity attenuates the influence of FTO variants on obesity risk: a meta-analysis of 218,166 adults and 19,268 children. PLoS Med 8: e1001116, 2011.*

**Reducing Sugary Drinks Could Reduce Obesity:** Recent studies have shown that, for some people, limiting the consumption of sugar-sweetened beverages leads to weight loss or reduced weight gain. Over the past few decades, the prevalence of obesity has increased significantly, leading many researchers to seek behavioral changes that might have played a causal role in the development of this public health epidemic. During that time, a dramatic rise in the consumption of sugar-sweetened beverages, such as some sodas, and sports, energy, and juice drinks, has paralleled the rise in obesity. Based on this observation, many scientists believe that sugar-sweetened beverage intake has contributed to the obesity epidemic and could be a potential target behavior for obesity prevention intervention strategies—an idea that was tested in a series of recent studies.

In one study, researchers sought to determine if genetics could influence whether sugar-sweetened beverage consumption affects risk for obesity. The scientists took advantage of existing data sets, collected as a part of three large-scale health surveys, which included physical, behavioral, and other characteristics from more than 30,000 U.S. men and women of European ancestry. They examined variations within 32 regions of each person's genome, which previous analyses have shown to be associated with body mass index (BMI, a measure of weight relative to height). The three health surveys also detailed sugar-sweetened beverage consumption, allowing the researchers to tease apart any association between these genetic and behavioral factors in obesity risk. The combined results from all three surveys were significant and clear: for individuals with many genetic risk variants predisposing them to obesity, there was a relatively greater association between consuming sugar-sweetened beverages and subsequent increases in BMI, particularly at higher levels of consumption.

In another study, researchers examined the effects in adolescents of an intervention substituting sugar-sweetened beverages with non-caloric beverages in their homes. The study included overweight and obese teenagers who regularly consumed sugar-sweetened beverages. For 1 year, participants received an intervention strategy designed to reduce intake of sugar-sweetened beverages in their homes: delivery of non-caloric beverages, motivational telephone calls with their parents, and periodic check-in visits. At the end of the year, sugar-sweetened beverage consumption was significantly reduced compared with a control group that did not receive the intervention. In addition, BMI increased less in the intervention group than in the control group during the intervention. A year after the intervention stopped, there was no longer a difference between groups overall in BMI, but Hispanic adolescents from the intervention group still showed less of a BMI increase. These results add to previous data suggesting a link between sugar-sweetened beverage consumption and excess weight gain, which may be greater in some individuals.

*Ebbeling CB, Feldman HA, Chomitz VR, et al. A randomized trial of sugar-sweetened beverages and adolescent body weight. N Engl J Med 367: 1407-1416, 2012.*

*Qi Q, Chu AY, Kang JH, et al. Sugar-sweetened beverages and genetic risk of obesity. N Engl J Med 367: 1387-1396, 2012.*

*A study on an intervention involving reducing sugar-sweetened beverage consumption in younger children, not funded by NIDDK, was published in the same journal as the preceding studies. In this intervention study, conducted in the Netherlands, researchers reported strong evidence that replacing sugar-sweetened beverages with non-caloric substitutes in schools significantly reduces weight gain in children (N Engl J Med 367:1397-1406, 2012), findings that complement those from the above U.S. studies.*

## DISEASES ASSOCIATED WITH CHILDHOOD OBESITY

**Increased Gallstone Disease in Obese Children and Adolescents:** Researchers have identified another health consequence of obesity in youth—increased risk for gallstones. A common, costly, and often painful condition in adults, gallstones were thought to be rare in children and adolescents. However, as with other adult diseases that are now developing at earlier ages in parallel with the childhood obesity epidemic, gallstones may also become more prevalent in youth. Previous studies had suggested a link between obesity and gallstones in children, as is the case in adults, but those studies had analyzed only a few individuals. To investigate further, researchers in the current study reviewed the electronic health records of over 500,000 youth, ages 10 to 19, who were participating in the Kaiser Permanente Southern California Children's Health Study. The researchers assessed whether the children and adolescents were underweight, normal weight, overweight, moderately obese, or extremely obese, and they identified 766 youth who had gallstone disease diagnosed within 2 years of enrollment in the study. The results showed that being overweight is associated with increased risk for gallstone disease, and that obesity and extreme obesity further heighten this risk. Gallstones were more common among girls than boys, particularly among obese girls, a finding that mirrors the increased risk for gallstones among obese women. The researchers also found that Hispanic youth were more likely to have gallstones than were individuals of other races/ethnicities. These results highlight the importance of further research to address both obesity and health disparities in childhood. Additionally, this study can inform current medical practice, as pediatricians may need to be increasingly aware that their young patients, particularly those who are obese, may develop gallstone disease.

*Koebnick C, Smith N, Black MH, et al. Pediatric obesity and gallstone disease. J Pediatr Gastroenterol Nutr 55:328-333, 2012.*

# *The Weight of the Nation –*
## *The NIDDK Collaborates on HBO Obesity Project*


One-third of American adults are obese. Another third are overweight. How did this happen? And how can we, as a nation, return to a healthy weight?

To help illustrate the answers—and to show the science of obesity and NIH's efforts to combat the obesity epidemic—the NIDDK and other components of the NIH collaborated with HBO and major research and health organizations to develop *The Weight of the Nation*, a documentary series and public education initiative that spotlights this urgent public health problem.

"If we don't succeed in turning this epidemic around, we are going to face, for the first time in our history, a situation where our children are going to live shorter lives than we do," said NIH Director Dr. Francis Collins, who appears in the full-length films. "It takes diverse and rigorous research to understand the causes of obesity and to identify interventions that work in the real world. The results from federally funded research, as seen in these films, can help to prevent and treat obesity and its complications."

The project consists of four documentary films originally aired on HBO in May 2012; a 3-part series for families; 12 short films, including one on NIH; and a nationwide community-based outreach campaign.

An HBO crew spent 2 days on the NIH campus, documenting how researchers are trying to understand, prevent, and treat obesity. "When it comes to America's health, there are problems which must be confronted, even if they make us uncomfortable, frighten us or are so daunting we don't know where to begin," said HBO producer John Hoffman, who previously worked with the NIH on HBO's *The Addiction Project* and *The Alzheimer's Project*. "With *The Weight of the Nation*, HBO is again proud to stand together with the NIH, as we step forward to…hopefully reverse the obesity epidemic."

HBO also filmed several NIDDK grantees at universities, as well as people struggling with obesity and its associated diseases, representatives from local governments, health care providers, and many others.

Leaders of the NIH Obesity Research Task Force from the NIDDK; the National Heart, Lung, and Blood Institute; the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development; and the National Cancer Institute provided scientific guidance for *The Weight of the Nation* films and screening kits, which are available in English or Spanish. Health centers, community groups, and others who sign up on the HBO web-site will get copies of the films and guidance on how the project can assist in their organizations' weight-control efforts. The films can be viewed online for free on the HBO web-site.

For more information, please visit the HBO web-site at *http://theweightofthenation.hbo.com* and the NIH project web-site at *http://www.nih.gov/health/NIHandweightofthenation/*

# SCIENTIFIC PRESENTATION

## Childhood Obesity Prevention Through Solution-oriented Research

*Dr. Thomas N. Robinson*

*Dr. Thomas N. Robinson is the Irving Schulman, M.D. Endowed Professor in Child Health, Professor of Pediatrics and of Medicine in the Division of General Pediatrics and the Stanford Prevention Research Center at Stanford University School of Medicine, and Director of the Center for Healthy Weight at Stanford University and Lucile Packard Children's Hospital. Dr. Robinson received both his B.S. and M.D. from Stanford University, and his M.P.H. in Maternal and Child Health from the University of California, Berkeley. He completed his internship and residency in Pediatrics at Children's Hospital, Boston and Harvard Medical School, and then returned to Stanford for post-doctoral training as a Robert Wood Johnson Clinical Scholar.*

*Dr. Robinson's "solution-oriented" research is largely experimental in design, including school-, family-, and community-based randomized controlled trials to test the efficacy and/or effectiveness of theory-driven, behavioral, social, and environmental interventions to prevent and reduce obesity, improve nutrition, increase physical activity and decrease inactivity, reduce smoking, reduce children's television and media use, and demonstrate causal relationships between hypothesized risk factors and health outcomes. At the February 2012 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, Dr. Robinson shared his perspective on research approaches and findings from studies he has conducted. The following are highlights from his presentation.*

**Solution-oriented Research: A Complementary Approach**

The predominant paradigm for health science research, Dr. Robinson noted, is to identify the underlying causes of and risk factors for disease, with the ultimate goal of using that knowledge to develop strategies for treatment and prevention. This "problem-oriented" research paradigm has produced numerous successes that have improved health, but it inherently emphasizes a somewhat reductionist view of complex diseases and does not always produce the evidence, rapidly enough, to address key questions for clinical and public health practice: "what works, and how to do it?" In his presentation, Dr. Robinson offered a complementary research approach. Multi-factorial conditions such as obesity, he argued, do not necessarily require a detailed understanding of their causes in order to develop effective treatment or preventative strategies. "Solution-oriented" research focuses on identifying the causes of improved health, positive outcomes, and reduced risk for disease.[1] This conceptual shift could have a significant impact on how a scientist generates hypotheses and studies. Solution-oriented research is based on the assumption that it is not always necessary to know the causes of a problem first in order to determine how to prevent or treat it effectively. This approach recognizes that the preceding, causal, or

[1] *Robinson TN and Sirard JR. Preventing childhood obesity: a solution-oriented research paradigm. Am J Prev Med 28: 194-201, 2005.*

contributing factors for complex diseases may no longer exist or may not be susceptible to change. Solution-oriented approaches focus on testing the efficacy of intervention strategies; such research questions are likely to be more relevant for clinical and public health practice and policy, and may shorten the time needed to translate research findings to improved public health.

Obesity is a condition well suited for solution-oriented research. Scientists generally accept that weight gain results from a disruption in energy balance, but numerous factors (*e.g.*, biological, social, psychological, economic, policy) likely have contributed to today's high levels of obesity. While research may help illuminate each individual factor's contribution to obesity, efforts to test treatments or prevention strategies, even in the absence of such knowledge, may ultimately yield more practical results. For example, in children, screen time (*e.g.*, television viewing, videotape viewing, video game use) is linked to obesity, but the reasons for this connection are unclear. Potential hypotheses for this link include: the effects of advertising, increased food consumption while viewing television, and the reduction in physical activity accompanying screen time. Most previous studies were observational, and study designs were complicated by inaccuracies in measuring screen time. Furthermore, even if a study proved that screen time contributes to obesity, that knowledge would not provide information on what to do, that is, how to reduce screen time or whether screen time changes will improve weight. The complementary approach would be to test an intervention to limit screen time and measure the resulting effects on the study participants' weight. This experimental approach was conducted by Dr. Robinson's research team. They found that in third- and fourth-grade students over a 6 month span, the average reduction of several hours of screen time per week led to approximately half the increased body mass index (BMI, a measure of weight relative to height) and waist circumference as was observed in children who did not alter screen time viewing.[2] Thus, the solution-oriented intervention reduced obesity in children, without knowing the specific causal factors linking screen time to obesity. In another study, Dr. Robinson and colleagues extended their analyses to younger children and assessed effects of screen time reduction over 2 years. In a trial with 4 to 7 year-old children with high BMIs, reducing screen time by 50 percent, compared to a control group, led to sustained improvement in BMI over a 2 year period.[3]

**Stealthy Interventions, Healthy Outcomes**

Can the idea of health benefits alone motivate children to change their behavior? Perhaps in some cases, but improved health may not be a potent factor driving many to become more physically active or to reduce calorie intake. Dr. Robinson proposed that a study design in which the target behavior of the intervention is intrinsically motivating, but its "side effects"—such as increased physical activity, reduced sedentary behavior, or dietary changes—would improve health. These types of solution-oriented "stealth interventions" could improve obesity-related behaviors without the appearance of health education.[4] To test this concept, Dr. Robinson's research team explored using dance in pre-adolescent girls as a fun target behavior, involving

[2] *Robinson TN. Reducing children's television viewing to prevent obesity: a randomized controlled trial. JAMA 282: 1561-1567, 1999.*

[3] *Epstein LH, Roemmich JN, Robinson JL, et al. A randomized trial of the effects of reducing television viewing and computer use on body mass index in young children. Arch Pediatr Adolesc Med 162: 239-245, 2008.*

[4] *Robinson TN. Save the world, prevent obesity: piggybacking on existing social and ideological movements. Obesity 18: S17-S22, 2010.*

participation in a group, cultural awareness, and the thrill of performance. They first tested whether hip-hop and other types of dance programs could improve BMI in fifth-graders, mostly of African American and Mexican American ethnicities. After 12 weeks, the girls who participated in the dance program maintained their BMIs, whereas BMIs rose in those who took standard physical education classes during the same time period. In a separate study, an intervention that included a culturally tailored dance program (hip-hop, step, and traditional African dance) and reduced television viewing time was compared with a nutrition education program in African American girls from California. Over a 12-week period, the BMIs and waist circumferences of the girls from the dance program and reduced television viewing group increased about half as much as those of the girls from the nutrition education group.[5] When the trial was extended to 2 years, they encountered challenges in program implementation, and BMIs did not change significantly. However, a number of obesity-related factors did improve in the girls in the dance and reduced television viewing group, including total cholesterol, LDL ("bad") cholesterol, depressive symptoms, and fasting insulin levels.[6]

Dr. Robinson described results from another "stealth intervention" trial—after-school sports programs for overweight children. Many aspects of team sports are highly motivating for children: participating on a team, receiving mentoring, developing friendships with young adult coaches, displaying skills to friends and family, and even having the opportunity to wear a uniform—"you'd be amazed how motivating shin guards are," Dr. Robinson explained. In the study, overweight children either participated in a soccer program or received health or nutritional education. After 6 months, the children who played soccer had significantly reduced BMIs, as well as increased physical activity measures, compared with those enrolled in the education programs. These findings show that although the contribution of each potential motivating factor is unknown, after-school team sports programs for overweight children could serve as effective interventions for weight control.[7]

**Taking Stealth Interventions to the Next Level**

Any obesity prevention or treatment strategy will require individuals to change their behaviors, but what will motivate people to act? The answer is likely different for each person. Dr. Robinson posits that because social and ideological movements drive many people to enact changes within their lives, they may provide opportunities to design stealth interventions for obesity prevention and treatment. Many such movements motivate large numbers of people to act: environmental sustainability/climate change, food safety, human rights/social justice, animal protection, and cause-related fundraising, to name just a few areas for potential stealth interventions. The issue of environmental sustainability/climate change inspires many people to change their behaviors, for example, walking or biking instead of driving. In the example of cause-related

[5] *Robinson TN, Killen JD, Kraemer HC, et al. Dance and reducing television viewing to prevent weight gain in African-American girls: the Stanford GEMS pilot study. Ethn Dis 13: S65-S77, 2003.*

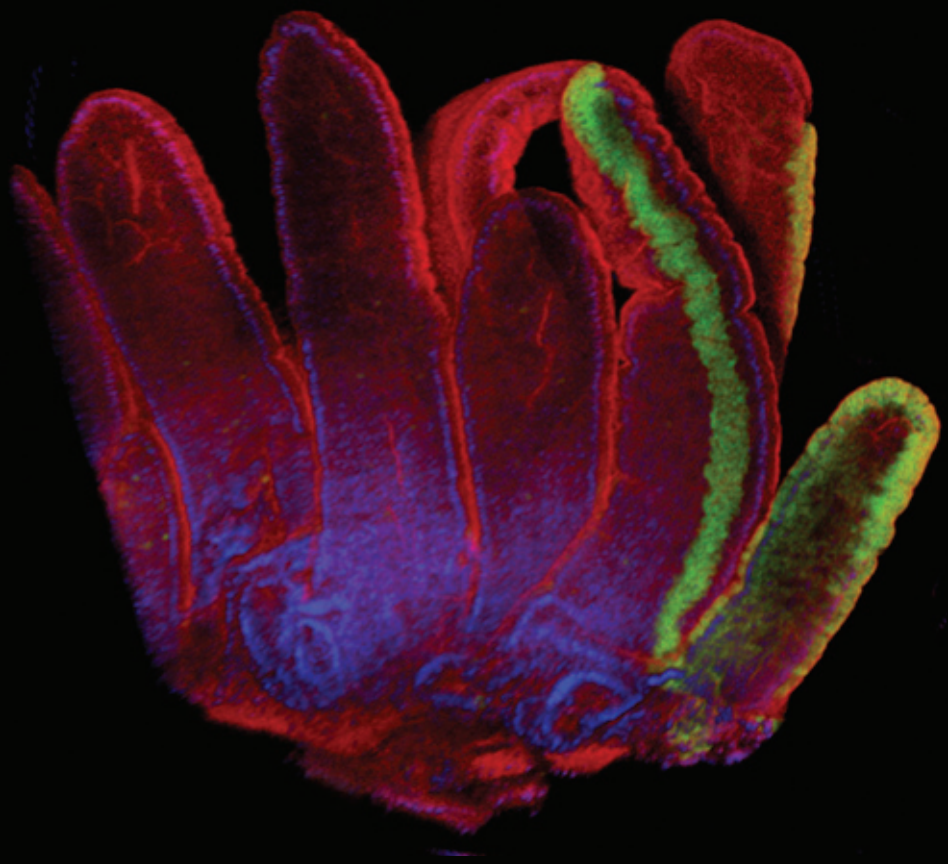
[6] *Robinson TN, Matheson DM, Kraemer HC, et al. A randomized controlled trial of culturally tailored dance and reducing screen time to prevent weight gain in low-income African American girls: Stanford GEMS. Arch Pediatr Adolesc Med 164: 995-1004, 2010.*

[7] *Weintraub DL, Tirumalai EC, Haydel F, Fujimoto M, Fulton JE, and Robinson TN. Team sports for overweight children: the Stanford Sports to Prevent Obesity Randomized Trial (SPORT). Arch Pediatr Adolesc Med 162: 232-237, 2008.*

fundraising, people may train for and participate in races, such as half-marathons, to raise money for charities. In both examples, improved health is not the motivating factor behind the behavior change, but it is a side effect. Dr. Robinson's current research investigates whether these and other social and ideological movements could be co-opted to serve as sources of effective interventions.

**Conclusions**

In presenting his team's ongoing research, Dr. Robinson makes the case that solution-oriented research, particularly through stealth interventions, could identify obesity prevention and treatment avenues for motivating people to change their individual behaviors. He proposes a litmus test for solution-oriented research: a study should only be done if the researchers know what to conclude from any possible result (positive, negative, or null) and if the findings may change intervention strategies to address obesity (or other public health problems). Dr. Robinson makes clear that problem-oriented research has enormous value; solution-oriented research adds an alternative and complementary methodology for tackling complex public health challenges like childhood obesity.



Intestinal stem cells from a specific lineage (green) travel up finger-like projections called villi on the surface of the mouse intestine to replace cells that were lost following injury from radiation. This type of stem cell complements the functions of other types of stem cells present in the intestine, which are involved in repopulation during normal cell turnover. This image was captured using a technique called three-dimensional confocal reconstruction, which allows the visualization of gene expression.

*Image courtesy of Dr. Manuel Amieva, Dr. Calvin Kuo, and Dr. Kelley Yan, Stanford University.*

# Digestive Diseases and Nutrition

*Digestive diseases are among the leading causes of doctor visits, hospitalizations, and disability in the United States each year. These conditions span a wide spectrum of disorders that affect the gastrointestinal (GI) tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. In 2004, more than 35 percent of all emergency and outpatient hospital visits—some 100 million—were associated with a diagnosis of a digestive disease.[1] While some digestive diseases are common and others quite rare; collectively, they exact a significant toll on public health in terms of their effects on quality of life, years lost due to premature death, and costs associated with hospitalization and pharmaceutical and surgical interventions. To reduce the public health burden associated with digestive diseases, NIDDK-supported scientists are vigorously pursuing research to better understand how widespread these diseases are across the United States and in specific population groups, to identify the causes of these diseases and how they progress, and to test new interventions for prevention and treatment of these costly diseases, including drugs, surgery, and behavior modification.*

Inflammatory bowel diseases (IBD), which include Crohn's disease and ulcerative colitis, are marked by destructive inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. These diseases often strike early in life, with a peak age of onset in adolescence or young adulthood. Treatment may require surgery, including removal of the affected region of the intestine. Scientists are investigating the complex interactions among the genetic, environmental, immune, microbial, and cellular factors that contribute to the development of IBD. The continued discovery of predisposing genetic variations, potential autoimmune and microbial influences, and new methods to grow intestinal tissue in cell culture will help catalyze the design of novel therapeutic strategies. Research on controlling intestinal inflammation has potential benefits not only for patients with IBD, but also for those at risk of developing colorectal cancer.

Diseases of the stomach and intestines include some of the most common digestive diseases, such as peptic ulcer disease, which is typically caused by an infection with the bacterium *Helicobacter pylori*, or use of non-steroidal anti-inflammatory drugs. Stomach and intestinal disorders also include functional bowel disorders, which result in abdominal pain and altered bowel habits. For example, irritable bowel syndrome (IBS) causes pain and constipation or diarrhea. IBS more frequently affects women, who may display a different range of symptoms and respond differently from men to pharmacologic treatments for the disease. While diet and stress contribute to this disorder, its underlying causes are unknown. Gastroesophageal reflux disease, in which stomach acids rise up into the esophagus, is a common functional bowel disorder that can lead to a condition known as Barrett's esophagus. This condition, in which cells lining the esophagus turn into an intestinal type of cell, is associated with a heightened risk of esophageal cancer, which is one of the cancer types still on the rise in the United States. Gastroparesis is another

[1] *Everhart JE, editor. The burden of digestive diseases in the United States. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. Washington, DC: U.S. Government Printing Office, 2008; NIH Publication No. 09-6443.*

functional bowel disorder, which is characterized by delayed emptying of food from the stomach, resulting in nausea, vomiting, and abdominal discomfort. A common cause of gastroparesis is diabetes, which is thought to damage nerves leading to the stomach and controlling movement of food; however, many cases are of unknown origin. Fecal incontinence, or impaired bowel control, is another bowel disorder that poses a major public health burden, particularly in the elderly.

Some digestive diseases can be triggered by the body's reaction to certain foods. For example, in individuals with celiac disease, the immune system reacts to the protein gluten—a component of wheat, barley, and rye—and damages the small intestine. This damage interferes with the ability of the intestine to absorb nutrients from foods and can result in chronic diarrhea, bloating, anemia, and, in children, slower growth and short stature. The only current treatment for celiac disease is maintenance of a strict gluten-free diet, which is difficult for many people. The greater challenge now facing patients and their health care providers is to improve methods capable of diagnosing celiac disease early, before damage occurs or other conditions develop. Recent and continued advances in the understanding of genes that predispose individuals to develop celiac disease may contribute to improved diagnosis in the future through genetic-based screening.

The microorganisms that inhabit the GI tract are important factors in maintaining or tipping the balance between digestive health and disease. These microbes can affect intestinal health in some surprising ways, depending on their interactions with each other, with intestinal cells, and with nutrients ingested by their human host. Scientists are gaining insights into the ways these GI microorganisms influence the development and function of the digestive tract and other systems throughout the body, such as those with immune and metabolic functions, as well as how the composition of the GI microbial community changes with factors such as age, geography, diet, and antibiotic usage.

The exocrine pancreas, which secretes enzymes required for digestion, is vulnerable to disorders such as acute and chronic pancreatitis, and their complications. Common causes of pancreatitis may include gallstones, heavy alcohol use, inherited genetic factors, drugs, and other causes. In all forms of pancreatitis, digestive enzymes attack the pancreas from within, causing inflammation, loss of function, and severe pain. Research has elucidated genetic and other factors contributing to pancreatitis that may lead to ways to treat or prevent this disorder.

The liver is an organ within the digestive system that performs many critical metabolic functions, including processing and distribution of nutrients such as fats. When the liver is functionally compromised by disease, serious adverse effects on health can occur, which sometimes lead to complete liver failure. Some liver diseases primarily affect children, such as biliary atresia (a progressive inflammatory liver disease), while others generally affect adults, such as a form of nonalcoholic fatty liver disease (NAFLD) or nonalcoholic steatohepatitis (NASH). In recent years, however, NAFLD has been increasingly diagnosed in children in the United States as well, concurrent with rising overweight and obesity. While some forms of liver disease are caused by viral infection, such as hepatitis B and C, or by genetic mutations such as alpha-1 antitrypsin deficiency, others arise from diverse factors such as autoimmune reactions, drug toxicity, and other triggers, some of which are unknown. Many liver diseases, such as chronic hepatitis B and C, place individuals at elevated risk for developing liver cancer. A healthy liver is necessary for life, and the only treatment for end-stage liver disease is a liver transplant. Because the number of livers available from deceased donors is limited, research is critical to identify liver disease early, find methods to preserve liver function in people with liver disease, and develop new treatment options, including transplants performed with liver tissue from living donors.

The number of Americans who are overweight or obese has risen dramatically in recent decades and is now at epidemic levels. Obesity is associated with numerous diseases, including type 2 diabetes, heart disease, and cancer. Multiple factors contribute to obesity. As scientists elucidate the molecular, genetic, and environmental factors that influence

appetite, metabolism, and energy storage, they are identifying potential avenues for the development of new intervention strategies to promote safe, long-term weight loss. In addition to new pharmacologic interventions for obesity, existing bariatric surgical techniques are being evaluated for their long-term impacts on weight loss and well-being. Investigators are also continuing research to help people achieve healthy lifestyles that include physical activity and improved diet. (Additional information on NIDDK-supported research endeavors focusing on obesity is provided in the Obesity chapter.)

Other nutrition-related disorders under investigation involve specific, inherited alterations in nutrient metabolism. NIDDK-supported research has enhanced knowledge of how these nutritional disorders develop, and how they can best be treated.

## NEW INSIGHTS ABOUT GUT BACTERIA AND HUMAN HEALTH

**Long-term Diet Determines Which Bacteria Reside in the Gut:** In their quest for greater understanding of how microbes inhabiting the body affect human health and wellness, scientists have found an association between individuals' long-term diets and the microbes that predominate inside their intestines. The current study analyzed the community of bacteria (microbiota) that populate the intestine and the effect of diet on different types of bacteria. For the first study, designated as "COMBO," a group of 98 healthy volunteers were asked to complete 2 questionnaires—1 asking for information on their diets over a long period of time (Food Frequency Questionnaire), and another asking the volunteers what they had eaten recently (Recall Questionnaire). The volunteers also provided stool samples, which contained bacteria from the intestines. The scientists then assessed the nutrients consumed by each of the volunteers by analyzing the dietary information from the questionnaires, and they identified the bacterial species within the gut microbiota by sequencing the bacterial DNA from the stool samples. Analyses of the DNA showed the gut microbiota of the volunteers contained two primary clusters of bacterial types found in the intestine, known as enterotypes—the *Bacteroides* enterotype and the *Prevotella* enterotype—with one of the two identified as dominant for each volunteer. By comparing dietary nutrients and bacterial species, the scientists determined that long-term dietary composition was associated with enterotypes. The *Bacteroides* enterotype was highly associated with a diet high in animal protein and saturated fat, whereas the *Prevotella* enterotype was associated with a high-carbohydrate diet. Ten of the volunteers also participated in a study called "CAFE," in which they ate a controlled diet while living in a hospital environment. All of the CAFE participants had been identified in the COMBO study as having dominant *Bacteroides* enterotypes, associated with diets high in animal protein and saturated fat. Half of the volunteers were given the high-fat/low-fiber (*Bacteroides*) diet, and half were given a low-fat/high-fiber (*Prevotella*) diet. Changes in microbiota composition were seen within 24 hours of starting a new type of diet, as detected by changes in the collective microbial genomes. Although significant, none of these changes in microbiota composition resulted in lasting changes in the volunteer's enterotype during the 10 days of the CAFE study. These studies indicate that, while dietary components over the short-term affect gut bacterial populations, long-term dietary composition determines the dominant enterotype of bacteria in the human intestine. This research has demonstrated an association between long-term diet and gut bacterial enterotypes. If future research finds that gut enterotypes, like dietary patterns, are associated with particular diseases, these results could have important implications for treating diseases through long-term dietary interventions to produce a healthy gut enterotype.

*Wu GD, Chen J, Hoffmann C, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science 334:105-108, 2011.*

**The Intestinal Microbiome Revealed as a Source of Human Genetic and Metabolic Diversity:** A recent study found that the composition of bacterial species that populate the human gut—the gut microbiota—evolves with age, particularly in the first years of life, and it differs among people from diverse geographic regions, potentially reflecting varying nutrition. Scientists

sequenced the gut microbiomes (microbiota DNA) of healthy individuals of different ages from the Amazonas of Venezuela, the African nation of the Republic of Malawi, and metropolitan areas of the United States to determine whether differences could be discerned in the diversity of bacterial communities and in the metabolic and nutritional functionality of the genes they contained. Microbiome DNA was obtained from fecal samples donated by members of the study cohort, which included parents, children, siblings, and identical and fraternal twins. A broad spectrum of information was obtained from the analysis of the microbiome data. Of particular importance, microbiota bacterial diversity increased with age in all populations, and bacterial species composition evolved from an infant microbiota into an adult microbiota during the first 3 years of life. In addition, the repertory of microbiome genes involved in vitamin biosynthesis, carbohydrate metabolism, and other metabolic functions also changed with age and differed among the countries. There were greater differences in bacterial community composition among the children than among the adults, and there were significant differences in the types of bacteria represented by the microbiomes of the three geographically representative populations. The greatest differences among populations were seen between the United States and the other two countries, in terms of their bacterial capacities for metabolizing vitamins, carbohydrates, proteins, and other substances, which closely reflect dietary patterns in these countries. This study reveals significant differences in the gut microbiome among young children and adults and among cultures with different diets, underscoring the importance of considering microbiome contributions in studies and nutrition-related policies involving human development, nutrition, physiology, and the impact of westernization.

*Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. Nature 486: 222-227, 2012.*

**"Friendly" Bacteria Compete with Disease-causing Bacteria in the Intestine:** In health, trillions of microbes (the microbiota) inhabiting the human gastrointestinal tract co-exist with their host in a relationship that is beneficial for both host and microbiota. However, when disease-causing (pathogenic) bacteria invade the gastrointestinal tract, they can disrupt this symbiotic relationship. Research scientists have recently uncovered important events that occur during pathogen infection.

Certain types of *E. coli* bacteria cause severe diarrhea when they infect the intestine. In a study exploring the invasion of hosts by virulent bacteria, research scientists used mice that were infected with a type of bacteria that mimic human *E. coli* infection, as a model system of the infection process. Intestinal infection requires that the invading pathogen compete with resident bacteria of the gut for nutrients and space. The symbiotic residential bacteria, known as commensals, make their home in the surface layer of the intestine called the outer mucosa. When pathogens invade, they begin synthesizing and secreting virulence factors that disrupt the outer and inner mucus layers of the intestine. Burrowing through the damaged mucosa, the pathogens colonize the unoccupied niche of intestinal epithelial cells where they cause damage and inflammation. In this study, the researchers infected different groups of mice with the pathogenic bacteria; one group had been raised in the absence of normal bacteria (germ-free) prior to infection, and the other group had been conventionally raised. They found that the conventionally raised mice were able to clear the infection, but the mice raised germ-free were not. Also, analysis of pathogen virulence factors revealed that they are important in conventionally raised mice for the early stage of infection, but production of these factors diminished later in infection, forcing the pathogens from their cellular niche back into the intestine's mucosa. There, the pathogens must compete with the commensals for a diet of simple sugars that they, the pathogens, require. However, in this environment the commensals tend to have the competitive advantage. Commensals that grow best on simple sugars successfully compete with the pathogens for this food. In fact, previous research found that infection by these pathogens alters the microbial community to promote growth of this type of commensals; the current study shows that these commensals then provide the benefit of devouring the food needed by the pathogens. Other types of commensals that are less picky about which

sugars they will eat do not out-compete the pathogens when mice are fed a normal diet of multiple types of sugars, but when faced with a diet of only simple sugars, they too will compete for this food, starving the pathogens and clearing infection. The knowledge that infection and clearance of intestinal pathogens is the result of both expression of virulence factors and a competition for nutrients indicates that diet or probiotic approaches may be explored for future treatment of intestinal infections.

*Kamada N, Kim Y-G, Sham HP, et al. Regulated virulence controls the ability of a pathogen to compete with the gut microbiota. Science 336:1325-1329, 2012.*

**Diet High in Milk Fat May Promote Harmful Intestinal Bacteria and Inflammation:** Scientists have shown that mice with a pre-existing genetic susceptibility to intestinal inflammation fed a diet high in saturated fats from milk have altered intestinal microbial communities that occur along with changes in bile acid composition, altered immune function, and increased intestinal inflammation. Inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis, are thought to result from a complex interplay between genetic and environmental factors. The rising incidence of these conditions in recent decades, together with studies showing increases in IBD in immigrants relocating from low-prevalence to high-prevalence countries and their children, point to a growing influence of environmental factors, such as diet. The dynamic communities of intestinal microbes, which are profoundly interdependent with humans in terms of metabolizing dietary nutrients, have received more attention in recent years for their potential contributions to human health and diseases such as IBD. In this study, scientists fed mice for 3 weeks on a diet high in fat—either milk fats, lard, or safflower oil from plants—which mimicked the fat levels found in Western diets, and compared them to mice fed a low-fat diet. They first looked to see the effect of these diets on the types and abundances of microbes present in the stool using genetic sequencing. All of the high-fat diets reduced the diversity of microbes present compared to the low-fat diet. In mice fed the high milk-fat diet, the scientists observed a "bloom" or explosion in the number of a particular bacterium, called *Bilophila wadsworthia*—a bacterium often detected in illnesses such as appendicitis and other types of intestinal inflammation. The researchers also examined mice with a genetic susceptibility to develop intestinal inflammation (colitis) due to deficiency of the gene encoding *Il10*, which is part of the immune system. These mice showed increased colitis when fed a milk-fat diet compared to the susceptible mice fed diets high in fats from plants or low in fat, or compared to mice without the genetic risk that were fed the high milk-fat diet. The genetically susceptible mice fed high milk-fat also had altered immune functions and more abundant levels of *B. wadsworthia* and taurocholic acid, a form of bile acid in which these bacteria thrive. Of note, the byproducts of bacterial metabolism of these bile acids and other substrates can injure and breach the inner protective barrier of the gut, leading to inflammation and damage. This pioneering work in mice connects the dots between genetics, the immune system, diet, and microbes to outline a compelling picture of how these factors may be interacting in the development of human intestinal inflammatory conditions such as IBD. While these findings require replication in humans, they offer a glimpse into the future of how these diseases might be treated or prevented in susceptible individuals through dietary and/or microbial means.

*Devkota S, Wang Y, Musch MW, et al. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice. Nature 487: 104-108, 2012.*

**Early Exposure to "Friendly" Microbes Protects Against Inflammatory Bowel Disease (IBD) and Asthma:** Diseases such as IBD and asthma are believed to occur, in part, because of inappropriate immune responses to "friendly" bacteria. However, exposure early in life to these bacteria appears to modify disease responses. In a study designed to explain these apparent contradictions, scientists have used mouse models to detect the relationships of microbes and immune cells to IBD and asthma. The scientists began their study by looking for pro-inflammatory cells called iNKT (invariant natural killer T) cells in tissues that line the intestines and

lungs of mice. These iNKT cells have been associated with inflammation related to ulcerative colitis (UC), a major form of IBD, and asthma. A greater number of iNKT cells were seen specifically in the intestinal linings and lungs of mice that were raised in germ-free conditions (GF mice) compared to mice that were raised in an environment of "friendly" bacteria, SPF (specific pathogen-free) mice. Also, the high number of iNKT cells was observed to remain constant for life. All of the study mice were given a chemical substance that mimics UC by inducing inflammatory symptoms. The GF mice responded with far greater sensitivity to UC induction, suffering severe weight loss and higher death rates compared to the SPF mice. To see if inflammation could be prevented in adult GF mice, the adult mice were introduced to "friendly" bacteria before UC induction, but this had no effect on disease development. However, when pregnant mice were exposed to "friendly" bacteria shortly before delivery, the offspring were protected against UC induction, confirming that protection against UC is a time-sensitive phenomenon that is acquired in the presence of "friendly" bacteria early in life.

This type of protection was also demonstrated in a mouse model of asthma. CXCL16 is a pro-inflammatory protein that regulates iNKT cells. In their search for a mechanism underlying susceptibility to UC and asthma, the scientists analyzed the concentration of CXCL16 in samples of mouse blood. The analysis revealed that concentrations of CXCL16, as well as iNKT cells, were significantly elevated in GF mice compared with SPF mice, and that iNKT cells were reduced in the intestines and lungs of mice when CXCL16 interaction with iNKT cells were blocked by antibody interference. Scientists have shown with this study that very early exposure to "friendly" bacteria is necessary to protect against inflammatory responses associated with UC and asthma in mice, and that this protection is enduring. Also, the mechanism for disease sensitivity is dependent on CXCL16 stimulation of iNKT cell inflammatory responses. Although this study was conducted in mice, the mouse system explored and its human counterpart are very similar, and it is expected that these findings will be relevant to developing new treatments for UC and asthma in humans.

*Olszak T, An D, Zeissig S, et al. Microbial exposure during early life has persistent effects on natural killer T cell function. Science 336:489-493, 2012.*

**Early Antibiotic Exposure Changes Gut Microbes and Fat Mass in Mice:** A group of scientists has found that even low-dose antibiotic exposure in young mice, similar to that given to farm animals raised in the United States, can dramatically alter the types of microbes present in the gut, as well as specifically increase fat mass. Antibiotic use is high in the United States, for both therapeutic purposes in humans and at subtherapeutic doses to boost growth in farm animals. However, there is some concern about the long-term effects of antibiotic use, not only because of the development of antibiotic-resistant bacteria (so-called "super bugs"), but also for other health effects of such exposures. Antibiotics are known to alter the delicate balance of microbes in the human gut, which is interconnected with energy balance and susceptibility to obesity. Scientists set out to explore how subtherapeutic antibiotic exposure in young animals might alter the gut microbial community, metabolism, and fat mass. They used as their experimental model young mice that had just been weaned. They gave the mice a 7-week course of antibiotics in their drinking water that was equivalent to doses used in the agricultural industry and compared them to mice given no antibiotics. While the mice exposed to antibiotics, as a group, did not differ in overall weight or growth during this period from mice without the exposure, X-ray scans revealed heftier fat mass in the antibiotic-treated mice. Mice given the antibiotics also had elevated levels of a hormone synthesized in the gut that stimulates fat cells. A microbial census taken by analyzing DNA present in the mouse stools and intestinal samples showed that, although antibiotic treatment did not affect the total number of microbes present, it altered the proportions of specific bacterial types. For example, antibiotic treatment was associated with elevations in bacteria called *Firmicutes*, which had been found to be elevated in another mouse model that is genetically prone to obesity. In the colon, major increases were observed in short-chain fatty acids, a product of complex carbohydrate metabolism by bacteria that can be used by colon cells for energy or can be absorbed into the circulation and stimulate fat tissue formation.

The liver also showed altered levels of gene activity associated with fat metabolism. Although the mice given antibiotics consumed the same amount of food as their non-treated counterparts, their fecal pellets showed less caloric content wasted, suggesting that their altered microbial community was more capable of extracting calories from the diet. This study provides a mechanism for the increased mass of farm animals given low-dose antibiotics. It also suggests that even low-level antibiotic exposure in young animals, and potentially humans, may come with an increased risk of obesity by increasing the numbers and activity of gut microbes that are more efficient at harvesting energy from the diet.

*Cho I, Yamanishi S, Cox L, et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature 488: 621-626, 2012.*

**Gut Microbes Transmit Susceptibility to Nonalcoholic Fatty Liver Disease Progression:** Researchers have shown how changes in the bacteria in the gut play a surprising role in the progression of nonalcoholic fatty liver disease (NAFLD). NAFLD is the liver's manifestation of metabolic syndrome, a group of risk factors that together increase the risk for cardiovascular disease and type 2 diabetes. In some people, the disease takes a relatively benign form, with excess fat accumulation in the liver, while in others, the disease progresses such that fat accumulation is accompanied by inflammation, liver damage, and scarring, called nonalcoholic steatohepatitis, or NASH. The cause of progression to the more severe form of the disease is still not well understood. However, in light of the direct connection between the intestine and the liver through the portal vein, scientists wondered if gut microbes, and the human host's microbial response system, might play a role in progression of this disease. Starting with the human immune system, the research team focused on inflammasomes—complexes of immune proteins involved in sensing microbes and triggering responses to those that are harmful. Mice that were genetically modified to lack some inflammasome components developed NASH when fed a special diet, showing that these human immune factors play a key role in putting the brakes on progression of this disease. But, even more interesting was that when these mice with genetic defects in microbe-sensing and severe NASH were housed together with "normal" mice without any genetic alterations or liver disease, the normal mice also developed the severe form of liver disease. The scientists concluded that a unique mix of intestinal bacteria in these mice lacking proper microbe-response machinery may have been transmitted to the normal mice, carrying the susceptibility to develop severe liver disease with it. They next sequenced genes characteristic of different types of bacteria from the intestines of the mice to identify the species present. They found a few species in particular that were present in unusually high numbers compared to intestinal bacteria in the normal mouse intestine. They also showed how these intestinal bacteria might bring about NASH by releasing their products into the blood and activating immune factors in the liver that lead to inflammation and damage. In addition, they examined another mouse model, this one genetically modified to be obese; these animals also "caught" the susceptibility to develop severe NASH from the inflammasome-deficient mice. These animals became even more obese and showed signs of increased insulin resistance. These studies have greatly expanded the understanding of how NAFLD's progression to NASH is related to intestinal microbes. More research is needed to tease out contributions by particular microbial species. However, this work may provide a basis for future antibiotic/probiotic therapies for individuals susceptible to developing NASH.

*Henao-Mejia J, Elinav E, Jin C, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature 482: 179-185, 2012.*

## GENETICS OF INFLAMMATORY BOWEL DISEASE

**Rare Genetic Variants Identified for Inflammatory Bowel Disease:** New technologies have enabled scientific researchers to uncover important rare genetic mutations associated with inflammatory bowel disease (IBD). Crohn's disease (CD) and ulcerative colitis (UC), the two major forms of IBD, are complex diseases attributed to errant immune responses caused by a

combination of genetic variants and environmental factors. Some variants are unique to either CD or UC, while others are shared. Using genome-wide association studies (GWAS) technology, as of November 2012, scientists had identified 163 genomic regions with variants that contribute to IBD. The variants that are known, however, account for only a small portion of IBD disease risk, and scientists have not yet identified the disease-associated gene within many of the implicated genomic regions. Scientists from several IBD research consortia and teams around the world have now used pooled "next-generation" sequencing technologies to examine 56 genes located in CD-associated genomic regions that had been identified by GWAS. Using pooled DNA samples from 350 CD patients and 350 healthy volunteers collected by the NIDDK IBD Genetics Consortium, IBD risk-related gene regions were sequenced using high-throughput sequencing technology. The scientists developed computer software to analyze the IBD sequencing data, which led to discovery of important rare variants. They further examined these variants in over 16,000 people with CD and 12,000 with UC, in comparison with more than 17,000 healthy individuals. A highly significant variant that confers IBD protection was identified in the *CARD9* gene. Two additional rare protective variants were identified in the *IL23R* gene and one in the *CUL2* gene. Five CD risk variants were identified in the *NOD2* gene. Two of the *NOD2* variants are more common in people of Ashkenazi Jewish ancestry—a population that is at high risk for CD—and one of the two variants is found only in the Ashkenazi population. In addition, associations in other gene coding regions were identified. This research has led to the discovery of multiple genetic variants conferring disease risk or protection in a number of IBD-associated genes previously identified by GWAS. These variants will provide valuable insights into the mechanisms by which these genes influence susceptibility to IBD. In addition, the identification of the *CARD9* variant, which offers disease protection, has the potential to lead to new therapeutic models for IBD prevention and treatment.

*Rivas MA, Beaudoin M, Gardet A, et al. Deep resequencing of GWAS loci identifies independent rare variants associated with inflammatory bowel disease. Nat Genet 43:1066-1073, 2011.*

**New Crohn's Disease Gene Variants Identified in Ashkenazi Jewish Population:** Scientists have discovered new genetic variants associated with Crohn's disease (CD) while investigating the Ashkenazi Jewish (AJ) population's high risk for this disease. The AJ, who are of Eastern and Central European descent, have a two- to four-fold greater risk of developing CD than Europeans of non-Jewish descent. Since the most frequent CD susceptibility variants discovered thus far in the AJ population are similar to those found in non-Jewish populations, those variants do not account for the higher risk for people of AJ heritage. For this study, scientists used new methods and technologies to identify unique genetic variations in this high-risk population. To mine the genetic information of this at-risk group, scientists gathered the largest number of AJ CD cases that has been assembled to date. This was accomplished by combining samples from several previous genome-wide association studies (GWAS), including the NIDDK IBD Genetics Consortium. To confirm the ancestry of the study participants, the researchers used genetic analyses to distinguish AJ ancestry from other populations, including non-Jewish Europeans and other Jewish populations. With additional analysis, the scientists were able to further distinguish AJ participants who had different degrees of AJ heritage—100 percent, 75 percent, or 50 percent, for both separate and combined analyses. The researchers then compared genomic data from AJ individuals with and without CD to identify genetic variants associated with this disease, and also compared genomic data from AJ study participants to data from non-Jewish individuals of European descent. These studies resulted in the discovery of five novel regions associated with CD in the AJ population, and the confirmation of several previously identified variants. The new and confirmed variants account for 11.2 percent of the total genetic variance for CD risk in the AJ population. This research demonstrates the complementary value of genetic studies in the Ashkenazi Jewish and other at-risk populations. The newly defined CD variant regions provide the basis for further studies of the biological pathways responsible for CD and may result in novel treatments.

*Kenny EE, Pe'er I, Karban A, et al. A genome-wide scan of Ashkenazi Jewish Crohn's disease suggests novel susceptibility loci. PLoS Genet 8: e1002559, 2012.*

## MECHANISMS OF LIVER AND BILIARY DISEASES

**Hepatitis B Virus Infection Control by Early Immune Cell Sensing:** Scientists have shown how an immune cell called the natural killer T cell mounts an early defense against hepatitis B virus infection by sensing modified fat molecules produced by infected liver cells. While most healthy adults clear the hepatitis B virus (HBV) on their own, some groups such as children are more vulnerable to chronic infection. The immune system has two main types of defenses against pathogens like HBV: innate immunity, which includes early cellular responders that attack invaders, and adaptive immunity, which involves activation of other cell types and the production over several days of specific antibodies against the invaders. Natural killer T (NKT) cells occupy a unique niche by responding early to pathogens as part of innate immunity, but then activating other cells that participate in both types of immune defense. Scientists set off to investigate what type of role these NKT cells might play in early defense against infection by HBV, with experiments in mice and in isolated liver cells. Because HBV does not naturally infect mouse liver cells, the scientists infected mice using a modified vector to deliver the HBV genome. As soon as 1 day after infection, NKT cells became activated in the liver, but not elsewhere. Using mice that were genetically modified to lack NKT cells and then infected with HBV, the researchers showed that activation of other immune cells typically stimulated by NKT cells was diminished, resulting in higher viral levels, fewer antibodies, and more hepatitis. In cell culture, they showed that mouse and human liver cells infected with HBV were necessary for the NKT cells to become active. When the liver cells became infected with HBV, they produced unique fat molecules (lipids) that signaled their infection to the NKT cells, which responded by producing chemicals that mount a full immune response and play a role in clearing the infection. These findings suggest that NKT cells contribute to early immunity against and clearance of HBV by sounding an alarm that alerts the immune system to the viral intruder. This research points to potential approaches to preventing and treating chronic HBV infection through targeting these cells.

*Zeissig S, Murata K, Sweet L, et al. Hepatitis B virus-induced lipid alterations contribute to natural killer T cell-dependent protective immunity. Nat Med 18: 1060-1068, 2012.*

**Transparent Fish Illuminates Formation of Ductal Network:** Scientists have employed a see-through fish for their research into the molecular mechanisms underlying formation and loss of the network of ducts connecting the liver, gallbladder, pancreas, and intestine. A system of ducts carries bile from the liver to the intestine to aid in fat digestion, with some bile stored in the gallbladder. Additionally, this ductal network carries digestive enzymes from the pancreas to the intestine, where they aid in digestion and absorption of nutrients. Some congenital conditions in humans result from improper formation or loss of these ductal networks. Two recent studies of these ductal networks utilized the zebrafish—a common animal model for research due to its ease of breeding and its transparency, which allows organ systems to be visualized directly.

In one study, scientists used a genetic screen and a fluorescently labeled marker to identify factors that regulate liver development in zebrafish larvae. They found that a gene, called *snapc4*, was important for liver development. The *snapc4* gene codes for a protein, similarly named snapc4, which regulates other genes with respect to whether they are on (expressed) or off. Livers of zebrafish with mutated (defective) *snapc4* were much smaller than normal. Further investigation of these mutants showed blocked transport of lipids consumed by the fish from the intestine to the gallbladder, indicating impaired functioning of the biliary duct network. By closely monitoring development of the larvae after fertilization, they observed that the biliary duct network initially formed, but the cells then died by a process called apoptosis and disappeared later in development. Additionally, the team found that the defective snapc4 protein was impaired in

its ability to bind with another protein called snapc2 in a larger functional complex, suggesting that both proteins are important for maintaining the bile duct network. The *snapc4* mutant zebrafish larvae showed features similar to the human disorders known as biliary atresia and vanishing bile duct syndrome, which are marked by biliary duct destruction from the disappearance of differentiated biliary epithelial cells through apoptosis. Future research could help determine if snapc4 and snapc2 play a role in this disorder.

Another research team used the zebrafish to investigate development of the ducts connecting the liver, pancreas, and intestine. They zeroed in on a gene called *sox9b*, which encodes a protein, sox9b, involved in regulating gene expression (although different from the factor identified in the other zebrafish study). *Sox9b* itself is expressed specifically in the ductal system. While researchers had previously identified the mouse version of *sox9b*, it had been difficult to study because even one copy of a mutated form of this gene is lethal for mice. Zebrafish, however, survive to adulthood with two genes encoding non-functional proteins, enabling studies of the effect of this gene on ductal development. Zebrafish with the non-functional form of *sox9b* exhibited impairment of the ductal system early in development, with blocked bile flow, as well as cells that should become liver and pancreatic ductal cells mistakenly taking on characteristics of the wrong cell types. Using fluorescently labeled markers for specific cell types, the scientists saw that the fish with this non-functional sox9b protein displayed a malformed network of ducts connecting the liver, pancreas, and intestine. Additional experiments showed that the sox9b protein is also important in maintaining ductal cell signaling through another pathway involved in early organ development through a protein called Notch. These findings shed new light on development of the ductal system and possible candidate genes (*e.g.*, the human *SOX9* gene) underlying human conditions with similar features such as biliary atresia or Alagille syndrome.

*Schaub M, Nussbaum J, Verkade H, Ober EA, Stainier DY, and Sakaguchi TF. Mutation of zebrafish Snapc4 is associated with loss of the intrahepatic biliary network. Dev Biol 363: 128-137, 2012.*

*Delous M, Yin C, Shin D, et al. sox9b is a key regulator of pancreaticobiliary ductal system development. PLoS Genet 8: e1002754, 2012.*

## LIVING DONOR LIVER TRANSPLANTATION RESEARCH

### Understanding Risk of Complications in Living Donors Undergoing Liver Transplantation:

A clinical study conducted at multiple sites across the United States has extensively described donor risk of long-term complications from living donor liver transplantation. Liver transplantation is the only option for those with end-stage liver disease, but the supply of organs available from deceased donors is severely limited relative to demand. Living donor liver transplantation can alleviate this problem, but potential risks to the donor must be investigated thoroughly prior to widespread use. For this purpose, the NIDDK's Adult-to-Adult Living Donor Liver Transplantation cohort study undertook an assessment of the incidence, severity, and natural history of donor complications from living liver donation. At 9 transplant centers throughout the United States, the investigators studied over 700 living liver donors over a 12-year period, the longest ever for a study of complications in these donors. They found similar rates of complications to previous, shorter-term studies, with 40 percent of donors having one or more complications and 19 percent experiencing multiple complications. The donors' estimated chances of disability, liver failure, or death after the procedure were 1 percent. Within the first few weeks after the transplant procedure, the most common complications were infections, excess fluid around the lungs, bile leaks, nerve damage, and bowel obstruction. Most of these complications resolved within 3 months after the procedure and, overall, 95 percent of complications were resolved within 1 year. Longer-term complications in the following months or even 5 to 6 years later included hernia, bowel obstruction, and psychological complications. Factors predicting a greater chance of complications included the donor's need for blood transfusion and low blood pressure during the procedure, features that are characteristic of more prolonged and complicated

surgery. Other factors that predicted specific serious complications included higher body weight, older age, and male gender. Surprisingly, among the nine transplant centers participating in the study, all of which had past experience in performing liver transplants, the extent of the center's experience in performing the procedure did not significantly predict donor complications. Further research will be needed to assess the long-term risk to donors of living donor liver transplantation as this procedure continues to evolve. These findings can be used to focus efforts on reducing the rate of complications and are invaluable in aiding the decision-making process of individuals who are considering becoming living liver donors and their loved ones.

*Abecassis MM, Fisher RA, Olthoff KM, et al. Complications of living donor hepatic lobectomy—a comprehensive report. Am J Transplant 12: 1208-1217, 2012.*

## UNDERSTANDING INTESTINAL IRON TRANSPORT

**Discovery of a New Protein Involved in Intestinal Iron Transport:** Scientists have discovered a new protein in rodents involved in intestinal iron transport, which may complement actions of other proteins required for facilitating absorption of this important nutrient. Iron is absorbed from food by cells of the small intestine and used for such essential functions as red blood cell production. In cases where insufficient iron is absorbed, anemia can result. Iron transport out of cells lining the intestine and into the circulation was thought to require an enzyme—hephaestin—that spans the cells' membranes. This enzyme oxidizes iron into a form that can be transported out of the intestinal cells. But, when scientists created a mouse with a mutated, inactive form of hephaestin, the animals were only mildly iron-deficient, suggesting another protein was compensating for the lost hephaestin. Scientists have since undertaken a search for a new enzyme with iron-oxidizing capabilities similar to those of hephaestin. They also considered the possibility of compensation by another enzyme known to oxidize iron, called ceruloplasmin. For this research, they used rat and mouse models with genetic mutations in these iron-oxidizing enzymes, as well as models with mild, diet-induced deficiencies in either iron or copper, because activity of these enzymes is known to increase when dietary iron levels are low, and both enzymes also happen to require copper in order to perform their functions on iron. Samples of intestinal cells taken from iron-deficient rats with elevated iron-oxidizing enzymes were broken open and processed to separate the cells' internal contents (the cytosol) from those in the cell membrane. Surprisingly, iron-oxidizing activity was detected in the cytosol, as well as in the cell membrane. Experiments with the rodents that had genetic mutations showed that iron was still oxidized, confirming the presence of a previously unknown iron-oxidizing enzyme in the cytosol of intestinal cells. This discovery of a new iron-oxidizing enzyme inside rodent intestinal cells, which may work in concert with the membrane-bound hephaestin to enable iron transport, deepens understanding of mammalian iron transport processes and related conditions.

*Ranganathan PN, Lu Y, Fuqua BK, and Collins JF. Discovery of a cytosolic/soluble ferroxidase in rodent enterocytes. Proc Natl Acad Sci USA 109: 3564-3569, 2012.*

# *NIDDK Grantee Dr. Thomas E. Starzl Wins 2012 Lasker Award*

**Dr. Thomas E. Starzl (left), winner of a 2012 Lasker award for his pioneering efforts in organ transplantation, is congratulated by NIDDK Director Dr. Griffin P. Rodgers (right) at the Lasker Awards ceremony on September 21, 2012.** ***Photo credit: Ellen Jaffe***

Dr. Thomas E. Starzl, Distinguished Service Professor of Surgery at the University of Pittsburgh School of Medicine and a long-time NIDDK grantee, received the 2012 Lasker-DeBakey Clinical Medical Research Award—shared with Dr. Roy Calne, University of Cambridge emeritus—for his work developing liver transplantation, an intervention that has restored normal life to thousands of people with end-stage liver disease.

Lasker awards are given for major advances in the understanding, diagnosis, treatment, cure, and prevention of human disease. Dr. Starzl performed the first human liver transplant. In addition to being a long-time NIDDK grantee, he is also a former NIDDK Method to Extend Research in Time (MERIT) awardee and has served on the NIDDK Digestive Diseases Advisory Board. He also earned a 2004 National Medal of Science.

"Dr. Starzl is a pioneer in the world of transplantation, and his work has saved thousands of lives," said NIDDK Director Dr. Griffin P. Rodgers. "This award is a most fitting recognition of his many years of unwavering commitment to teaching, research, and clinical practice."

Receiving the award on September 21, 2012, Dr. Starzl said, "Transplantation services are not provided by single individuals. The team is what counts, and it is on behalf of my research and clinical teams—first in Denver and then in Pittsburgh—that I accept this prize. And by the way, the prize could have gone to one of those courageous kidney, liver, or heart recipients who faced the great unknown in the early years and chose to run the uncharted gauntlet of transplantation instead of giving up. Win or lose, these were the heroes."

# *LiverTox: A New Online Resource for Information on Drug-Induced Liver Injury*


The NIDDK's Liver Disease Research Branch, in collaboration with the National Library of Medicine's Division of Specialized Information Services, has developed an online resource for information on drug-induced liver injury resulting from prescription and over-the-counter drugs as well as from complementary and alternative medicines such as herbals and dietary supplements. Called "LiverTox," this web-based resource provides up-to-date, accurate, and easily accessible information on the diagnosis, cause, frequency, patterns, and management of liver injury attributable to these agents.

Liver injury from medications, herbals, or dietary supplements has emerged as an increasingly important health problem in the United States. Although most cases of liver injury are mild and resolve quickly, some individuals develop liver injury so severe that it can lead to acute liver failure and, ultimately, transplantation or death. In the United States, liver injury due to drugs is the leading cause of acute liver failure, occurring with increasing frequency in recent years based on findings from the NIDDK's Acute Liver Failure Study Group.

One of the challenges in treating this form of liver injury is the accurate and timely identification of the drug(s) causing the injury so that steps can be taken to limit the damage. Different drugs or supplements can cause disparate patterns of liver injury that can sometimes mimic other forms of liver disease, making it difficult for physicians to recognize unless they first rule out all other potential causes of liver injury.

The creators of LiverTox set out to remedy this by providing a comprehensive source of information to aid health care providers and patients in diagnosing, and researchers in studying, liver injury due to specific drugs, herbals, or dietary supplements. The web-site serves as a centralized "one stop shop" for information relating to the prevention and control of drug-induced liver injury from multiple agents, as well as guidance on the diagnosis and management of this important cause of liver disease.

LiverTox has three major components: 1) an introduction and overview of drug-induced liver injury, 2) a series of individual drug records or monographs on specific medications, herbals, and dietary supplements describing their liver toxicity with specific case histories and a complete set of references, and 3) a case submission registry that allows users to report a case directly to LiverTox and which then can be forwarded to the U.S. Food and Drug Administration's Adverse Event Reporting System (MedWatch). The LiverTox database currently includes information on approximately 700 drugs or supplements available in the United States, and the current plan is to add 300 more over the next few years. Case reports of liver toxicity were collected from several sources, including the published scientific literature, the database of the NIDDK's Drug-Induced Liver Injury Network, and cases seen at the NIH Clinical Center.

LiverTox's creators envision it as a "living textbook" with ongoing updates and improvements. They will continue to draw upon the collective wisdom of the wider scientific and health care community by welcoming comments and information on all known drug- and supplement-related forms of liver injury from web-site users, in hopes of reducing these forms of liver injury in the future.

The LiverTox web-site is accessible at:
*http://livertox.nih.gov/*

# *Workshop Charts Progress and Promise of Pancreatitis Research*


In June 2012, the NIDDK convened a 2-day research workshop at the NIH campus in Bethesda, Maryland, on "Advances in Acute and Chronic Pancreatitis: From Development to Inflammation and Repair." The workshop provided a state-of-the-art update on a wide range of research efforts addressing acute and chronic pancreatitis and charted a course for advancing future research in this area.

Pancreatitis is a disease marked by inflammation of the pancreas; the inflammation results from aberrant activation of digestive enzymes. Normally, the pancreas releases digestive enzymes that subsequently become activated within the intestine to aid the digestion of food. In pancreatitis, the enzymes become activated while still within the pancreas, causing tissue damage and pain. Pancreatitis can be acute, with inflammation resolving within a few days, or chronic, involving long-term inflammation and tissue damage. A variety of factors can contribute to the development of pancreatitis, including genetics, gallstones, heavy alcohol use, and other causes. Currently, there are no cures or preventive therapies for pancreatitis. The NIDDK actively supports research on pancreatitis, including clinical trials such as the North American Pancreatic Study 2 and Study of Nutrition in Acute Pancreatitis.

Major drivers for organizing this timely workshop were current gaps and opportunities in pancreatitis research. The workshop was co-organized by NIDDK staff; two members of the NIDDK Advisory Council who are active in this area, Dr. Anil Rustgi, Chief, Division of Gastroenterology, University of Pennsylvania, School of Medicine, and Ms. Jane Holt, co-founder of the National Pancreas Foundation; as well as others in the external pancreatitis research community.

The objectives guiding this workshop were:

- To enhance understanding of pancreatic developmental and stem cell biology, acute and chronic forms of pancreatitis, genetics of pancreatitis, the link between the inflammatory pancreatic microenvironment and neoplasia, and pancreatic neurobiology;
- To identify new strategies in diagnosis, imaging, and therapy of pancreatitis;
- To foster collaborative and innovative interdisciplinary translational research; and
- To coalesce the meeting findings into a publication as a roadmap for future NIH-based initiatives.

Topics discussed over the 2 days ranged from basic research on pancreatic development and regeneration to mouse models of pancreatitis and new treatment strategies based on genetic susceptibility and environmental factors. Some participants described results from efforts supported by the NIDDK, such as studies conducted through the Beta Cell Biology Consortium and North American Pancreatitis Study 2. Presenters came from around the globe to share their findings, representing research institutions from the United States, England, Canada, Germany, and Spain. Participants also shared research resources, such as the "Pancreapedia" web-site, which is supported in part by the NIDDK (*www.pancreapedia.org*).

This productive workshop continues to bear fruit by guiding investigators toward promising future research directions in this area. Consistent with one of its objectives, a report summarizing the meeting was published in January 2013 in the journal *Gastroenterology* (144: e1-e4, 2013), in order to share knowledge and recommendations with the larger research community focused on advancing understanding and improving care for those with acute and chronic pancreatitis.

# STORY OF DISCOVERY

## *Alpha-1 Antitrypsin Deficiency—From Genes to Therapies*

NIH-funded research over the past decades has helped to decipher the genetic underpinnings and clinical manifestations of alpha-1 antitrypsin (AAT) deficiency, an inherited disorder associated with liver disease, as well as disease in other organs such the lungs. Although the disease is caused by a single abnormal gene, its manifestations vary greatly depending upon the specific mutation a person has, whether a person inherits copies of the abnormal gene from one or both parents, and also on other genetic and environmental factors. With knowledge gained from research has come an expanded understanding of AAT deficiency, the differences among its various forms, and development of new therapeutics based on this scientific foundation.

The AAT enzyme is synthesized in the liver and secreted into the bloodstream, where it is transported throughout the body to help protect against tissue damage. It plays a particularly important role in the lungs, where it prevents the breakdown of proteins in connective tissue that help the lungs remain flexible.

AAT deficiency is a genetic disorder in which the gene encoding AAT is mutated, resulting in lower levels of this protein in the blood and diminished AAT activity in the lungs. AAT deficiency is a major contributor to chronic obstructive pulmonary disease and emphysema due to lung damage. The lower levels of AAT in the lungs are a consequence of the retention of malformed AAT protein in the liver, where its accumulation can cause tissue damage. AAT deficiency is the most common genetic cause of liver disease in children and an uncommon but important cause of liver disease in adults, sometimes leading to chronic liver disease and liver cancer. Patients with AAT deficiency thus face potential health risks on two fronts: lung disease, due to insufficient circulating AAT, and liver disease, due to accumulation of AAT in this organ. Research has greatly increased scientists' understanding of the molecular processes involved in AAT deficiency, and recent studies have highlighted a promising new approach to addressing the sometimes life-threatening consequences of this serious condition.

**Molecular and Cellular Features of AAT Deficiency**

Since the mid-1960s, scientists have identified over 120 variants of the AAT gene. These variants are grouped into three categories based on the level of AAT they release into the bloodstream—normal, deficient, or virtually undetectable. About 100,000 Americans have the most severe form of AAT deficiency. In these patients, a significant fraction of the mutant AAT protein does not complete its journey from the interior of a liver cell, where it is assembled, to the cell surface, where it would normally be released into the bloodstream. Rather, the mutant protein forms polymers—chains of AAT molecules—which aggregate within liver cells at a site that acts as a checkpoint for quality control of proteins. The retention of polymerized AAT within the liver has two adverse consequences: first, the accumulation of AAT polymers results in tissue damage, including inflammation and fibrosis, in this vital organ; second, it results in lower levels of AAT in the bloodstream, which means that this protein cannot perform its important protective role of keeping in check the tissue-degrading enzymes in distal organs, especially the lungs.

**Clinical Characterization of AAT Deficiency**

The importance of AAT was originally recognized in studies of blood proteins in 1963, when scientists

# STORY OF DISCOVERY

found that some patients with emphysema lacked sufficient amounts of AAT in their blood. In 1969, other researchers observed that patients with a particular variant of the AAT gene had a high frequency of liver disease, including neonatal jaundice and cirrhosis. In addition, the patients had high concentrations of the variant AAT in their liver cells. These and other observations about the disease enabled fundamental research to begin uncovering underlying mechanisms—a prelude to the development of treatments.

Scientists supported by the NIH have focused vigorous research efforts on liver damage arising from AAT deficiency. Because not all people with variant AAT develop liver disease, researchers have searched for factors that might predispose some patients to be susceptible to or protected from this liver damage. To this end, in 1994, researchers grew skin cells from AAT-deficient individuals who had never suffered from liver disease and who therefore might be "protected." Similar cultures were made with cells from AAT-deficient individuals who had severe liver disease and were therefore considered "susceptible." While cells from both cultures accumulated the variant AAT protein, only the "susceptible" cells exhibited a delay in degrading this abnormal protein, suggesting that some AAT-deficient patients have alterations in the degradation pathway for the protein in their liver cells—alterations that may predispose them to developing liver disease.

**Possible Treatments for AAT Deficiency**

In the 1980s, scientists demonstrated that increasing a patient's levels of functional AAT by administering the normal form of the protein was feasible and beneficial. AAT-deficient patients achieved an increase in their blood levels of the normal protein following the intravenous transfusion of purified AAT from the blood of healthy individuals. Further research led to the FDA approval of a purified form of the enzyme for the treatment of AAT-related lung disease in 1987. Related therapies on the horizon are intravenous AAT augmentation products, inhalation delivery systems, and synthetic augmentation therapies. However, while these therapies for AAT-related lung disease are promising, they do not address the other manifestation of AAT deficiency: liver disease.

In the late 1980s, a study of mice engineered to produce a mutant human form of AAT secreted sufficient AAT into their bloodstream to protect them from lung disease but still suffered from a build-up of AAT in their liver cells and showed signs of liver damage. In 1989, additional research supported by the NIH substantiated that the aggregation of AAT protein within liver cells caused disease. Subsequent studies showed that patients with AAT deficiency sustain liver damage due to inflammatory immunological responses to the aggregated protein, and that the immunosuppressive drug cyclosporin A could help prevent AAT liver damage—a proof-of-principle for immune mechanism-based therapeutic approaches to AAT deficiency. All of these studies point to the accumulation of AAT as a key factor in the development of liver disease in AAT deficiency.

Building on these findings, more recent studies have focused on strategies to limit the accumulation of malformed protein in the liver to address the manifestations of AAT deficiency in this organ. One approach has targeted "autophagy"—literally, "self-eating"— the process by which a cell breaks down and recycles its components. In 2010, basic research studies demonstrated that, in liver cells, increased autophagy affords limited protection from damage caused by AAT aggregates. Treatment with the drug carbamazepine markedly reduced the amount

of accumulated protein in cultured cells by boosting autophagy activity, and this drug also reduced liver fibrosis in a mouse model of AAT deficiency. Notably, carbamazepine has been used as an anticonvulsant and mood stabilizer in humans for over 40 years and its safety and tolerability are well known. Using a system to screen large numbers of drugs, investigators are searching for agents that improve autophagy and reduce the AAT aggregates in cells. Targeting the autophagy pathway may be a promising strategy for future therapeutic approaches.

**Looking Forward**

Despite recent progress toward new therapies, the only effective treatment for liver failure due to AAT deficiency is liver transplantation, for which donor organs are severely limited. Therefore, there remains a need for alternative therapies that can treat or prevent the serious liver disease that often accompanies AAT deficiency. By combining basic research on cellular processes underlying disease with knowledge of existing therapeutics that target these processes, researchers are striving to identify promising treatments that may work for multiple diseases. While autophagy-enhancing drugs are a promising potential treatment for liver disease associated with AAT deficiency, further studies will be needed to test the benefits and risks of this treatment in pediatric and adult patients with this serious form of liver disease.

*The NIDDK supports a broad range of research related to liver disease from AAT deficiency in children and adults. One example is the Childhood Liver Disease Research and Education Network (ChiLDREN), which was created by joining the Cholestatic Liver Disease Consortium and the Biliary Atresia Research Consortium. ChiLDREN includes studies of AAT deficiency and is funded by the NIDDK with substantial support by the Alpha-1 Foundation. Through research on its collection of clinical data and biospecimens, this Network is poised to gain a better understanding of how AAT deficiency leads to liver disease, as well as to contribute to the development of new treatments for this condition.*

# PATIENT PROFILE

## Marcia Chichester

*Her "Brother's" Keeper—Two Friends Bound Together by Living Donor Liver Transplantation*

**Marcia Chichester (right) with Jason Donley (left)**

In 2011, Marcia Chichester received her registered nursing (RN) degree after working 21 years as a licensed practical nurse. To mark this important achievement, like other RNs before her, she participated in a pinning ceremony where someone special to her attached her RN pin. She chose her good friend and former co-worker Jason Donley to perform this honor. But, as Jason walked across the stage and attached Marcia's pin, what outside observers couldn't see was the deeper bond these two friends share—he was alive and well that day because of her selfless gift 6 years before.

**Faith and Friendship**

In the early 2000s, Marcia and Jason were both nurses at a hospital in Charlotte, Michigan. Marcia, then a single mom with two teenage sons and working two nursing jobs, offered to spend her day off to drive Jason to a doctor's appointment 3½ hours away to see a specialist who treated his primary sclerosing cholangitis or "PSC." PSC is a form of liver disease marked by inflammation and scarring of the bile ducts leading from the liver to the intestine, which causes bile to back up into the liver and damage the organ.

As a teenager, Jason was diagnosed with ulcerative colitis, and then was diagnosed with PSC after college. Both PSC and ulcerative colitis are forms of autoimmune disease that often occur together and run in families. Over time, PSC causes a yellowing of the skin called jaundice, pain along the right ribs, nausea, and weight loss. "I started glowing in the dark," he recalls. "It was hardest watching my family deal with it. My mom would lift my shirt up and put her arm next to my tummy to see how yellow I was. And if I was really yellow, she'd just go off and cry."

But the worst symptom for Jason was the intense and insatiable itching caused by the liver disease. "The itching will drive you insane," he recalls. "It's an itch you just really can't quench." The itching was intensified by sweating, so he was careful to maintain cool temperatures at home, take regular ice water baths, and minimize physical activity. Despite the challenges of living with PSC, Jason threw himself into his work, taking on extra shifts to distract himself from the itching. But, he knew that it was only a matter of time until the disease progressed to a point where he would need a liver transplant.

# PATIENT PROFILE

After that long trip together, Marcia and Jason became good friends, and, along with another co-worker, formed a bible study group. Marcia also continued to accompany him to all his out-of-town doctor's appointments. On one appointment in February 2005, Jason's doctor informed them that he would need a transplant soon and should consider living donor liver transplantation. A few years before, he and Marcia had learned about the procedure at a liver transplant orientation meeting at Northwestern University in Chicago, Illinois. At that meeting, they also heard about a clinical study on this procedure called the Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL), and they both remember being enthusiastic about the study. (See box at end of profile for more information on A2ALL.)

Jason considered a living donor transplant, but his immediate family members were not eligible to donate due to his siblings' own autoimmune diseases and the age of his parents. He also began the process of getting on the waiting list for a deceased donor organ, though his chances of receiving one in time were slim due to the high demand and the rules of the system used to allocate these organs. As Marcia watched Jason grow sicker, she read online about a woman who donated part of her liver to a non-relative. Marcia resolved to be Jason's donor, if possible, and discussed it with her family.

"Well, he was sick and going to die. And I told my boys, 'I might be a match'—they were 18 and almost 15 at the time," remembers Marcia. "They both said 'You've got to do it, Mom.' We're a family with a really strong faith, and we knew that if it was meant to be, it would work and everything would be fine." She informed the rest of her family, then visited Jason to tell him that she wanted to be his living donor. He was concerned about the impact of such a decision on his friend and her family, particularly her teenage sons, and discussed it with them to ensure all were supportive of the decision.

*Marcia resolved to be Jason's donor, and discussed it with her family. "He was sick and going to die. And I told my boys, 'I might be a match'," she remembers. "They both said 'You've got to do it, Mom.'"*

Jason chose to have the transplant performed at Northwestern, where Marcia underwent a series of tests to evaluate whether she could donate to him. They were a match for blood type, but a liver imaging test found some fat in her liver, which can be a marker of fatty liver disease and compromise functioning of the organ in both a potential donor and recipient. That made her ineligible to donate, but she was determined. "I went to my doctor and said 'How do I get rid of this?'," she recalls, referring to the liver fat. Her doctor recommended a weight loss program, as she was overweight at the time. She immediately went on a strict diet and rigorous exercise regimen in an effort to lose weight and reduce the fat in her liver.

Meanwhile, Jason was running out of time. Weeks later, he became very ill, and when they went to Northwestern for his appointment, Marcia asked to be tested again. The nurse warned her that if this second test was also positive for liver fat, she would not be allowed to be tested again. Again, Marcia was undeterred and insisted on a retest. Surprisingly, the second test found no fat in the liver, and she was permitted to complete the rest of the matching process for donating part of her liver to Jason. She met all of the criteria, and they set a date for the transplantation, deciding also to enroll in the A2ALL Study. The good news came just in time—Jason was expected to live only a few months without a liver transplant.

# PATIENT PROFILE

**Living Donor Liver Transplantation**

The liver is an organ with two lobes located in the abdomen beneath the ribs that is required for survival. Every day, it silently multi-tasks—processing nutrients, neutralizing toxins, and performing a number of other vital functions. It also shows a remarkable capacity for partial regeneration, or being able to regrow after injury. But when damage occurs to the point where liver function is severely compromised by disease, as was the case with Jason, the organ cannot regenerate adequately, and a liver transplant is the only option.

Initially, liver transplants were only performed using organs from deceased donors. However, because the need for livers for transplantation is far greater than the number of deceased organs available, alternative approaches were needed. Due to the liver's capacity to regenerate, living donor liver transplantation was a therapeutic possibility, and the first living donor transplant was performed in the late 1980s. Further refinements in living donor liver transplantation have improved the outcomes in both donors and recipients, although complications from the procedure still occur. As with any major surgery, there is a risk of death from living donor liver transplantation, and donor deaths have occurred, even at well-established transplant centers with experience performing the procedure. True to their Hippocratic oath of "first, do no harm," doctors are especially concerned with finding ways to prevent complications and death in the healthy living donor. Research, such as the A2ALL Study that Marcia and Jason decided to participate in, aims to provide insights into the risks and benefits of the procedure.

**A Complicated but Rewarding Journey**

In July 2005, Marcia and Jason, accompanied by their families and a few close friends, returned to the hospital at Northwestern for the living donor liver transplant procedure. He was now extremely ill—a vivid shade of yellow and gaunt, down to around 160 pounds on his 6-foot frame. The group arrived early and spent a day touring Chicago together. "We decided we were going to have one fun day and then if something happened, we would have that day to remember," recalls Marcia.

At the hospital the following day, both Marcia and Jason were being prepared for the surgery when they were informed that an emergency situation had arisen that required the attention of the same transplant team. The pair graciously agreed to delay their surgery until 5 days later, returning to Michigan in the meantime.

On July 18, 2005, Marcia, Jason, and their families held a prayer session before heading over to the now-familiar hospital at Northwestern. They were taken to separate rooms in the surgical ward and prepared for surgery. Over the next several hours, the transplant team replaced Jason's liver with the right lobe of Marcia's liver.

Within just a few days of the transplant, Jason recovered with remarkable speed as his new liver immediately began performing the vital functions that had been compromised by the PSC for so long. The day after surgery, 95 percent of his jaundice was gone, and he was walking the hospital hallway. For the first time in many years, his skin was no longer yellow, and the itching that had tormented him was gone. By the time of their 3-month check-up, both Marcia's and Jason's livers had regenerated to over 90 percent of their full size.

But the procedure was not without complications. As they recovered from their surgeries, their shared religious faith, along with the support of one another, friends, and family, would see them through some difficult times ahead. A few days after the surgery, Marcia was back in the hospital with a painful bowel obstruction. Now it was Jason's turn to act as his

# PATIENT PROFILE

"sister's" keeper, visiting her and vigilantly monitoring her charts and care until she recovered. By 1 month after transplantation, both Marcia and Jason were able to return home. But in the second month, Marcia experienced a hernia at the site of the surgery and was re-admitted to the hospital for surgery to repair the hernia. She experienced a second hernia 2 months later, requiring another surgery. Although the complications caused her to be out of work for 5 months, she took them in stride. "I was happier that I had the complications and not Jason because I could physically take it better than him," she says.

Jason made it through the first few years after transplant without any complications, though, like other organ transplant recipients, he was required to take medications that suppress his immune system to prevent rejection of his new liver. But, in March 2008, his liver enzymes spiked up. He was diagnosed with autoimmune hepatitis, and treated with additional immunosuppressive drugs. Jason continues to be on the drugs, the long-term use of which carries some risk of side effects, such as bone loss. So far, he has tolerated them well and shows no signs of rejecting the transplanted liver.

*"It's just something that had to be done. I decided I had to do it," Marcia says, adding "I would hope anyone would do it for someone who was going to die."*

Despite the complications along the way, neither Marcia nor Jason regret their decision to undergo what for him has been a life-saving procedure. Jason and his family are grateful to Marcia for donating part of her liver to him, a gift that represents a "huge extension on life." Jason explains that, "She is part of the family. They think the world of her." Marcia feels she has also benefitted from being a living donor. "The benefit is that I know that somebody's alive and that they wouldn't be alive if it wouldn't have been for me. That makes me feel good," she says. When people call her heroic, she responds with her characteristic humanity and resolve. "It's just something that had to be done. I decided I had to do it," she says, adding "I would hope anyone would do it for someone who was going to die."

**Helping Others Through Research**

Marcia and Jason have also given back to the community of donors and recipients undergoing living donor liver transplantation through their ongoing participation in the A2ALL Study at the Northwestern University site. At the time when they went through their procedures, doctors had limited knowledge about the risk of complications to the donor and recipient. Now, thanks in part to the A2ALL Study's findings, they have additional information that has allowed them to improve the procedure and better inform other donors and recipients of what they can expect, including types and chances of potential complications.

"That's why we got in the study, because we wanted to be able to provide information and to let people know more," says Marcia. Jason echoes that point of view. "I always look at medicine as: we're always practicing it. So the more people that are willing to be in a study so they can get that practice perfect, I'm all for it."

They continue to go to Chicago together every year for Jason's check-ups and to participate in the A2ALL Study. They enjoy reading about the Study's findings and maintain close relationships with the doctors and researchers. "You get to know your whole transplant team, and every time Marcia and I are back there, it's like a reunion," says Jason. "You get another surrogate family."

# PATIENT PROFILE

*"That's why we got in the study, because we wanted to be able to provide information and to let people know more," says Marcia. Jason echoes that point of view. "I always look at medicine as: we're always practicing it. So the more people that are willing to be in a study so they can get that practice perfect, I'm all for it."*

**Bound for Life**

Marcia and Jason are now bound for life—the scars on their abdomens serve as a reminder of their unique connection. Though they no longer work together and live an hour's drive apart, they continue to stay in close contact with each other and their families.

Since the transplant, Marcia has remarried and obtained her RN degree. She now works as an assistant director of nursing at a long-term care and rehabilitation facility. She enjoys spending time with her family, including her 1-year-old grandson, scrap-booking, and traveling the world with her husband. She also remains a strong advocate for living donor liver transplantation, taking advantage of every opportunity to share her experiences with others who are considering becoming living donors, even having "business" cards made with her contact information to distribute to doctors' offices.

Currently, Jason works as the clinical service director for a hospice company, helping care for people at the end of life and their families. He appreciates his new-found abilities post-transplant of renewed energy, eating foods he enjoys, and engaging in physical activity and breaking a sweat without fear of itching. He also enjoys pursuing his interests in photography, deer hunting, and traveling.

No matter where life's journey takes them next, Marcia and Jason share an unbreakable bond, to each other and to making the most out of their lives post-transplant.

# PATIENT PROFILE

## ADULT-TO-ADULT LIVING DONOR LIVER TRANSPLANTATION COHORT STUDY (A2ALL)

The A2ALL Study began in 2000 to study living donor liver transplantation (LDLT), at a time when use of the procedure was expanding despite the limited information available on its risks and benefits compared to the traditional deceased donor liver transplantation (DDLT) procedure. Chief among the study's objectives were to determine whether LDLT was as safe and effective as DDLT for the recipients, and to capture the rates and types of complications from the LDLT surgery, particularly those experienced by the donors. Currently, the Study is conducted by nine liver transplant centers with expertise in adult LDLT located around the country and a central coordinating center.

The Study has already succeeded in showing that LDLT is safe and effective, on par with DDLT, as a life-saving therapy for some patients, particularly those like Jason who would otherwise have to wait a long time for a deceased donor organ. "He is a perfect example of somebody who would have fallen off the waiting list curve," says Dr. Michael Abecassis, Director of the Comprehensive Transplant Center at Northwestern University Feinberg School of Medicine, the A2ALL site where Marcia and Jason's living donor liver transplantation was performed. "Having gone through the Study we can now feel even more confident about doing this type of transplant for this particular individual."

The Study's findings reflect the improvements in LDLT that have taken place in recent years as transplant centers gained more experience in performing the procedure. Dr. James Everhart, the Program Director at the NIDDK who spearheaded the A2ALL Study initiative, recalls, "When living donor liver transplantation first started, the recipients did not do as well. That has changed, and they now do as well as if they had received a liver from a deceased donor." Now, based on the Study's results, having an LDLT rather than a DDLT is advantageous for many patients by reducing their risk of dying while on the waiting list for an organ. "That was an important finding because, for the recipient, there's a real advantage to getting this surgery. It's almost exclusively because they're able to receive a donated organ earlier, rather than waiting for an organ from a deceased donor," says Dr. Everhart.

A2ALL has also achieved its objective to quantify the risk of complications to donors from the LDLT procedure. In a paper published in the *American Journal of Transplantation* and featured elsewhere in this chapter, A2ALL Study investigators described the risks and types of long-term complications for donors undergoing LDLT, in which they found that 40 percent of donors had one or more complications, a rate consistent with other national and international estimates.

"I think one of the best things about A2ALL is that now we can be a little more precise when we are communicating the potential risks, especially to the donor," says Dr. Abecassis. In 2005, when Marcia decided to give part of her liver to Jason, "we really did not have a number to give her, for example, for the risk of a hernia," which she experienced on two occasions. Dr. Abecassis is particularly struck by the willingness of Marcia and others to be living liver donors at a time when

# PATIENT PROFILE

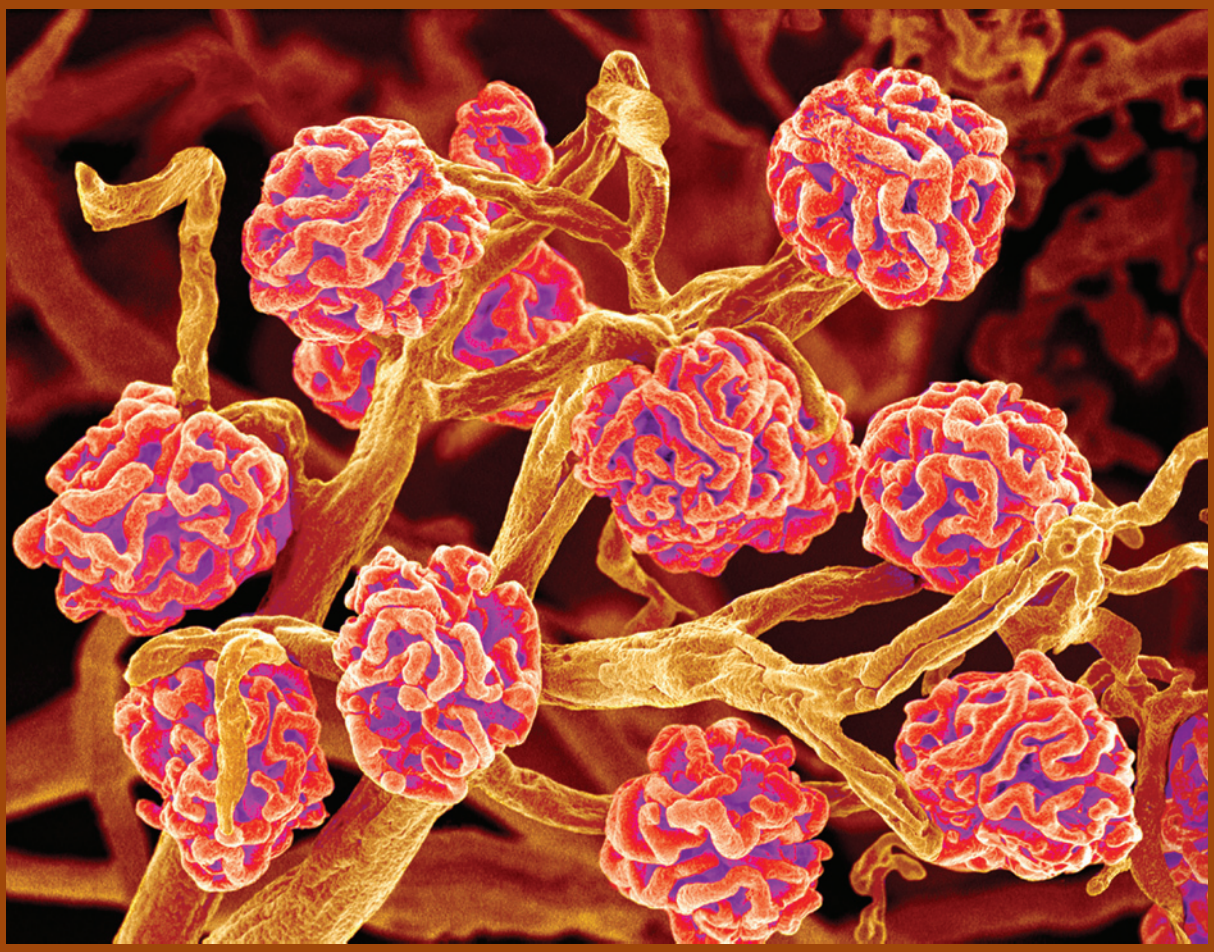
information about the risk of donor complications was so limited.

Now, because of the long-term efforts of those involved with A2ALL, the picture of LDLT's risks and benefits for donors and recipients is much clearer. "Because the study went on and we were able to follow patients who had the procedure at the beginning of the centers' experiences, we've now been able to say, instead of just a snapshot, here's the movie—this is when you're likely to get these types of complications, this is how severe they're likely to be, and this is how likely it is that those complications might get resolved," says Dr. Abecassis. "We have a lot more confidence now when we talk to potential donors and recipients about what is likely to happen."

Originally designed for 7 years, this highly productive study was funded for an additional 5 years to collect longer-term data on the liver transplant participants and their outcomes. As the Study comes to a close and the investigators consider ways to pursue additional unanswered questions about LDLT, they plan to continue mining the rich resource of long-term data they've collected.

Dr. Abecassis credits the collective power of the Study with enabling its important findings and improving transplant centers' knowledge of LDLT. "Because we were together in a consortium, and we were meeting on an ongoing basis, our program was able to learn certain things from our colleagues, and they learned other things from us. Sometimes, we just learned together from our collective experience. At a time when the procedure was evolving, this was undoubtedly a very valuable exercise." He adds, "This consortium has been particularly productive, not just in terms of academic productivity but in terms of collegial thinking. We're still learning from each other, and this experience continues to benefit our patients."

*Additional information on the A2ALL Study can be found online at: www.nih-a2all.org*



Glomeruli are clusters of small, looping blood vessels in the kidney that perform the first step in the body's removal of waste products, salts, and excess fluid from the circulation while retaining red blood cells and blood-borne proteins. Each kidney contains approximately one million of these tiny filtering units. This image shows a group of glomeruli and the larger vessels that deliver blood to them.

*Photo credit: Susumu Nishinaga/Science Photo Library*

# Kidney, Urologic, and Hematologic Diseases

*Diseases of the kidneys, urologic system, and blood are among the most critical health problems in the United States. They afflict millions of Americans and their impact is felt across the lifespan. To improve our understanding of the causes of these diseases, and to identify potential new treatments for them, the NIDDK supports basic and clinical research studies of the kidney and urinary tract and disorders of the blood and blood-forming organs. The overall goal of the NIDDK's research programs is to increase our understanding of kidney, urologic, and hematologic diseases to in order to enhance approaches to prevent and treat these serious conditions.*

Normal, healthy kidneys filter about 200 quarts of blood each day, generating about two quarts of excess fluid, salts, and waste products that are excreted as urine. Loss of function of these organs, either for a short period of time or as a consequence of a gradual, long-term decline in kidney function, represents a life-threatening condition.

It has been estimated that more than 23 million Americans have impaired kidney function—also called chronic kidney disease (CKD).[1] CKD has two main causes: high blood pressure and diabetes. The increases in obesity and type 2 diabetes in the United States in recent years—especially among children and adolescents—have grave implications for the Nation's health, as young people with these conditions are likely to face serious health complications at an earlier age than people who historically have developed these conditions later in life.

One feature common to kidney diseases arising from varying causes is the deposition of fibrotic scar tissue in the kidney. Research supported by the NIDDK has enhanced our understanding of the origin of this scar tissue, how it can impair kidney function, and how it might be prevented or treated.

CKD, especially if undetected, can progress to irreversible kidney failure, a condition known as end-stage renal disease (ESRD). People with ESRD require dialysis or a kidney transplant to live. In 2010, nearly 600,000 patients received treatment for ESRD: over 400,000 received either hemodialysis or peritoneal dialysis and almost 180,000 were living with a kidney transplant. Minority populations, particularly African Americans, Hispanic and Latino Americans, and American Indians and Alaska Natives, bear a disproportionate burden of CKD and ESRD. African Americans are nearly four times more likely to develop kidney failure than are non-Hispanic whites.[2] American Indians and Alaska Natives and Hispanic and Latino Americans have twice the risk for kidney failure as do non-Hispanic whites. In recent years, scientists supported by the NIDDK have uncovered important genetic clues that may play a role in health disparities related to kidney disease susceptibility and progression in minority populations.

The NIDDK supports a significant body of research aimed at understanding the biology underlying CKD. The NIDDK's chronic renal diseases program supports

[1] *Levey AS, et al. Ann Intern Med 150: 604-612, 2009.*

[2] *U.S. Renal Data System, USRDS 2012 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2012.*

basic and clinical research on kidney development and disease, including the causes of kidney disease, the underlying mechanisms leading to progression of kidney disease to ESRD, and the identification and testing of possible strategies to prevent development or halt progression of kidney disease. The NIDDK also supports studies of inherited diseases such as polycystic kidney disease, congenital kidney disorders, and focal segmental glomerulosclerosis; and immune-related kidney diseases such as IgA nephropathy and hemolytic uremic syndrome.

The NIDDK's National Kidney Disease Education Program (NKDEP) is designed to raise awareness about the problem of kidney disease and steps that should be taken to treat CKD and prevent kidney failure. NKDEP represents a major educational outreach effort to patients, physicians, and the public.

Urologic diseases affect people of all ages, result in significant health care expenditures, and may lead to substantial disability and impaired quality of life. The NIDDK's urology research program supports basic and clinical research on the normal and abnormal development, structure, function, and injury repair of the genitourinary tract. Areas of interest include the causes of and treatments for urological diseases and disorders such as benign prostatic hyperplasia, urinary incontinence, and urinary tract infections. As described in this chapter, NIDDK-supported research has identified a potential new treatment approach for urinary tract infections. Other disorders of the genitourinary tract, such as interstitial cystitis/painful bladder syndrome (IC/PBS) in women and men and chronic prostatitis/chronic pelvic pain syndrome in men, are also important components of the NIDDK's urology program. Recent research in an animal model has demonstrated that sex differences in pelvic pain exist. Additional areas of interest include research on treatments for kidney stones, such as shock-wave and laser lithotripsy to break up stones, and therapeutic approaches to inhibit their formation and growth.

IC/PBS is a debilitating, chronic, and painful urologic disorder. Based on a recent large national interview survey, it is estimated that 3.3 million (2.7 percent) U.S. women 18 years old or older have pelvic pain and other symptoms, such as urinary urgency or frequency, that are associated with IC/PBS.[3] Using a community-based epidemiological survey, researchers have estimated that 1.6 million (1.3 percent) U.S. men ages 30 to 79 years old have persistent urologic symptoms, such as pain with bladder filling and/or pain relieved by bladder emptying, that are associated with painful bladder syndrome.[4]

NIDDK-supported basic and clinical research on IC/PBS is focused on elucidating the causes of these conditions, identifying "biomarkers" that will aid diagnosis, and improving treatment and interventions. Ongoing epidemiologic studies will help refine prevalence estimates and demographics. These include the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) research network, which supports research designed to uncover the underlying causes of IC/PBS and to characterize the disease profiles in patients. A profile of a patient who is participating in the MAPP study appears in this chapter. Other studies include the Boston Area Community Health Survey (BACH), which seeks to identify patterns and risk factors for a range of urological problems, and the Olmsted County (Minnesota) Study, which is studying lower urinary tract symptoms in men.

Urinary incontinence is conservatively estimated to affect 13 million Americans, most of them women.[5] Many suffer in silence due to embarrassment and lack of knowledge about treatment options available. NIDDK-supported studies over the past several years have helped to advance knowledge about the efficacy of surgical treatment of urinary incontinence, as well as provide new insights into non-surgical alternatives. As researchers continue to investigate treatment options, an equally important challenge is to improve strategies for assessing both the impact of urinary incontinence and the effect of different diagnostic tools and interventions on patient outcomes.

---

[3] *Berry SH, et al. J Urol 186: 540-544, 2011.*

[4] *Link CL, et al. J Urol 180: 599-606, 2008.*

[5] *Urological Diseases in America. NIDDK, NIH Publication Number 07-5512, 2007*

The NIDDK's hematology research program uses a broad approach to enhance understanding of the normal and abnormal function of blood cells and the blood-forming system. Research efforts include studies of a number of blood diseases, including sickle cell disease, the thalassemias, aplastic anemia, iron deficiency anemia, hemolytic anemias, thrombocytopenia, and the anemia of inflammation and chronic disease. NIDDK-supported research has recently identified a potential new approach to mitigate the severity of two serious forms of anemia in an experimental model.

The NIDDK is also keenly interested in the basic biology of stem cells, including adult hematopoietic (blood) stem cells, which are needed for bone marrow transplants and may have broader application in gene therapy research. Studies have identified a protein involved in aging of blood stem cells. An additional priority of the NIDDK's hematology research program is the development of improved iron-chelating drugs to reduce the toxic iron burden in people who receive multiple blood transfusions for the treatment of diseases.

## CHRONIC KIDNEY DISEASE

**A Better Way To Estimate Kidney Function:** Researchers have recently shown that measuring creatinine and cystatin C—two markers for chronic kidney disease (CKD)—more precisely estimates kidney function than measuring either marker alone. Creatinine is a waste product from protein in the diet and the normal breakdown of muscle tissue. Cystatin C is released by cells throughout the body. Normally, the kidneys remove both creatinine and cystatin C from the blood, and they are excreted in the urine. As kidney disease progresses, however, the kidneys do this job less well, leading to increased levels of creatinine and cystatin C in the blood.

Within the kidney, the glomerulus performs the task of filtering waste products and excess salts and fluid from the blood. The "glomerular filtration rate," or GFR, is a measure of the kidneys' capacity to filter the blood. However, GFR is rarely measured outside of a research setting. It is most commonly estimated using a mathematical equation that incorporates, among other factors, an appropriate biomarker, usually the level of creatinine in the blood. Physicians and scientists have long known that the method of estimating GFR by measuring creatinine alone is imprecise, because creatinine levels can vary among individuals due to factors that are not related to kidney function, such as differences in muscle mass, malnutrition, or chronic illness. This imprecision can have negative consequences, such as incorrectly classifying patients as having CKD when they may not, leading to unnecessary treatment of healthy individuals. It can also fail to detect the decreased kidney function in patients who do have CKD and who would benefit from treatment. The new study found that using a revised calculation that incorporates both creatinine and cystatin C levels produced more accurate estimates of GFR over a broader range of kidney function and body size than estimates utilizing creatinine alone, and was less likely to be altered by other medical conditions. This research was conducted as part of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) study, which estimated kidney function in a diverse group of over 5,000 people from 13 studies.

Estimated GFR is routinely reported with the results of the most commonly ordered clinical blood tests. Thus, this CKD-EPI study may have important implications for routine medical care for adults. Furthermore, because there is no effective treatment to restore kidney function once it is lost, prevention and early detection of kidney disease are critically important approaches to prevent kidney failure. These results are an important step towards improving the certainty of CKD diagnosis.

*Inker LA, Schmid CH, Tighiouart H, et al. Estimating Glomerular filtration rate from serum creatinine and cystatin C. New Engl J Med 367: 20-29, 2012.*

**Health Risks from Chronic Kidney Disease Independent of Diabetes:** A meta-analysis of over 40 studies that enrolled over 1 million patients found that the presence of chronic kidney disease increases the risk of cardiovascular disease and death, and

that similar outcomes were seen in patients with and without diabetes.

There are approximately 23 million Americans with chronic kidney disease, and worldwide prevalence has been estimated to be between 10 and 16 percent of all adults. The most common cause of chronic kidney disease is diabetes, and the most common cause of death in patients with chronic kidney disease is cardiovascular disease. The current study examined patient data from 30 studies of the general population and high-risk patients and 13 studies of patients with chronic kidney disease in order to determine the risk of progression to kidney failure or cardiovascular disease and death in patients with chronic kidney disease. Specifically, the researchers were interested in learning whether the presence of diabetes in these patients had an impact on the likelihood of progression to kidney failure, cardiovascular disease, or death. When comparing patients with and without diabetes who had the same level of estimated kidney function, the risk of kidney failure, cardiovascular disease, and death were much the same in both groups.

Although patients with diabetes have an increased risk for cardiovascular disease and kidney failure, this study found that the relative risks of these outcomes were much the same irrespective of the presence or absence of diabetes when kidney function was included in the analysis. This observation highlights the association of poorer outcomes with reduction in kidney function and underscore the important role of kidney health as a predictor of outcomes independent of diabetes status.

*Fox CS, Matsushita K, Woodward M, et al. Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without diabetes: a meta-analysis. Lancet 380: 1662-1673, 2012.*

## GENETIC VARIATION AND KIDNEY DISEASE

**Genetic Variants Linked to Earlier Kidney Failure in African Americans:** Scientists have identified an association between variants in the *APOL1* gene and two measures of the severity of kidney disease in African Americans. In two separate studies, they found that *APOL1* gene variants are associated with the rate of decline in kidney function in African Americans and also with the age at which individuals begin hemodialysis to treat kidney failure.

These studies build on information learned over the past few years about the contribution of genetic factors to the increased risk of kidney disease in this population. Previous studies have shown that African Americans with two copies of certain variants of the *APOL1* gene are at increased risk of developing kidney disease. Indeed, these two variants, termed G1 and G2, are believed to explain much of the increased risk of non-diabetic kidney disease in this population.

One study examined biosamples from participants in the African American Study of Kidney Disease and Hypertension (AASK). The AASK study enrolled African American patients with mild kidney disease due to hypertension and found that treatment with an angiotensin-converting enzyme inhibitor was better than two other drug options at slowing kidney disease progression. The investigators asked whether *APOL1* and other gene variants were associated with an increased risk of worsening kidney disease in 700 AASK participants. They analyzed archived DNA samples and found that the presence of the G1 variant of the *APOL1* gene was associated with a faster decline of kidney function compared to study participants without this variant.

Another study examined over 400 African Americans with kidney failure and asked whether the presence of 1 or 2 copies of either *APOL1* variant was associated with a younger age at which the participants began hemodialysis, a therapy used to cleanse the blood of waste products and excess fluids and salts when the kidneys no longer function. The researchers found that African Americans with two copies of the G1 variant began hemodialysis at a significantly younger age (approximately 49 years old) than those with 1 copy of the variant (about 56 years old). People with 2 normal copies of the *APOL1* gene began hemodialysis at around 62 years of age.

These findings have important implications for understanding the differences in kidney disease progression across different populations. They also suggest the *APOL1* G1 variants may be a marker of an increased rate of kidney disease progression in African Americans. Therefore, early interventions to prevent progression of chronic kidney disease in this high-risk population may be of particular benefit.

*Kanji Z, Powe CE, Wenger JB, et al. Genetic variation in APOL1 associates with younger age at hemodialysis initiation. J Am Soc Nephrol 22: 2091-2097, 2011.*

*Lipkowitz MS, Freedman BI, Langefeld CD, et al. Apolipoprotein L1 gene variants associate with hypertension-attributed nephropathy and the rate of kidney function decline in African Americans. Kidney Int: 83: 114-120, 2013.*

**Gene Mutations Linked to Hypertension:** Scientists have identified mutations in two genes that play a role in the regulation of blood pressure and salt balance in a rare, heritable disease that causes high blood pressure (hypertension), a leading cause of chronic kidney disease and kidney failure. The researchers were studying a rare, inherited form of hypertension called pseudohypoaldosteronism type II, or PHAII. They analyzed genetic samples from 41 families with PHAII and identified mutations in either the *KLHL3* or *CUL3* genes in most. The proteins encoded by these genes, KLHL3 and CUL3, come together in kidney cells as part of a complex that targets other proteins for breakdown and recycling or other processes. The scientists hypothesize that these mutations inhibit this function of *KLHL3* and *CUL3*, thereby disrupting normal cellular processes. The researchers also noted that the hypertension associated with PHAII can be successfully treated with drugs that promote increased fluid excretion by acting on a protein that regulates the absorption of sodium and chloride in the kidney—and that the *KLHL3* and *CUL3* genes are expressed in the same region of the kidney as the drug-regulated protein.

This finding suggests that KLHL3 and CUL3 may be involved in helping to maintain fluid and salt balance by regulating how much fluid is retained by the kidneys' filtering system and how much is excreted in urine. A fuller understanding of the roles these proteins play in blood pressure maintenance and fluid and salt regulation in PHAII will provide further insight into the regulation of this delicate balance in these patients. It may also identify novel targets for the treatment of hypertension arising from other causes in the general population.

*Boyden LM, Choi M, Choate KA, et al. Mutations in kelch-like 3 and cullin 3 cause hypertension and electrolyte abnormalities. Nature 482: 98-102, 2012.*

## GENE REGULATION AND KIDNEY DEVELOPMENT AND MAINTENANCE

**New Insights into Kidney Formation:** New research indicates that the protein called Dicer plays an important role in kidney development by regulating micro-ribonucleic acid (miRNAs). The discovery of molecules known as miRNA has challenged the prevailing scientific thinking about the role of RNA in gene expression, the process by which information stored within DNA is decoded into messenger RNA (mRNA) which in turn is translated into a protein. Different genes produce different mRNAs, which code for different proteins. Both mRNA and miRNA are made up of a string of nucleic acids; however, miRNA is much shorter than mRNA. miRNAs can interact with mRNA to block the ability of mRNA to be translated into protein, thereby adding another level where gene expression can be regulated. Mammalian genomes contain a large and diverse family of miRNAs. It is now believed that miRNAs might affect one-third of all human gene expression.

The protein Dicer plays a key role in processing miRNA from its initial long pre-miRNA form to its shortened, mature form. Scientists sought to determine the role, if any, of Dicer and miRNAs in regulating kidney development. To investigate Dicer's potential role, mice were genetically altered to remove Dicer function from cells that form the nephron and ureteric bud-derived collecting duct system—two compartments of the mammalian kidney. Kidneys were removed prior to or shortly after birth and

evaluated for developmental abnormalities. Lack of Dicer function in cells destined to become part of the nephron led to increased cell death, incomplete nephron formation, and smaller kidneys. Removal of Dicer function from the ureteric bud resulted in the development of kidney cysts (sacks of fluid that replace healthy tissue). In addition, Dicer removal disrupted normal structural features of the ureteric bud.

This study provides evidence that Dicer, and presumably the miRNAs processed by Dicer, have distinct and critical regulatory roles within different components of the developing kidney. Further investigation of the role of miRNAs might shed new light into understanding how the number of functioning nephrons in the kidney is determined and the underlying causes and development of cystic kidney diseases.

*Nagalakshmi VK, Ren Q, Pugh MM, Valerius MT, McMahon AP, and Yu J. Dicer regulates the development of nephrogenic and ureteric compartments in the mammalian kidney. Kidney Int 79:317-330, 2011.*

**Defective DNA Repair and Chronic Kidney Disease:** Mutations in a protein that is part of the cellular machinery that helps maintain the genome and repair DNA damage might contribute to chronic kidney disease, as well as to a rare cystic kidney disease called nephronophthisis (NPHP)-like ciliopathy.

In a recent report, scientists identified mutations in the gene that encodes the FAN1 protein as a cause of karyomegalic interstitial nephritis (KIN), a rare and slowly progressive chronic kidney disease marked by fibrosis and cells with massively enlarged cell nuclei. The FAN1 protein is part of a multi-protein complex that works to repair damage to DNA, which is contained in the cell nucleus. Specifically, this complex breaks the inappropriate chemical bonds that sometimes link one chromosome to another. These inter-chromosomal cross-links can form following exposure to toxins, or as a result of normal metabolism and aging. Such cross-links, if not repaired, can cause enlarged nuclei, prevent gene activation and cell division, and ultimately lead to cell death.

The scientists showed that FAN1 mutants had an impaired ability to remove DNA cross-links in patients with KIN, and suggested that this diminished capacity to repair DNA damage was an important factor in progressive kidney disease. Evidence for the key role of FAN1 was also seen in animal studies. In the zebrafish, lower levels of FAN1 caused a defect similar to KIN, with diminished DNA repair, cell death, and kidney cysts.

Further support for the importance of DNA repair was observed in a well-characterized rat model of kidney disease arising from high blood pressure, in which the extent of DNA damage in the kidney was correlated with kidney failure. This observation supports the hypothesis that faulty DNA repair may be an important underlying cause of kidney disease arising from various causes.

DNA damage has been shown to be associated with exposure to toxins, and the kidney—as an organ whose primary function is to remove waste products from the blood—is likely exposed to levels of toxic agents that are higher than those in most other organs. This study identifies defective repair of DNA damage in the kidney—and the accumulation of this damage, subsequent cell dysfunction, and cell death—as an important cause of fibrosis and subsequent loss of kidney function in patients with KIN. As fibrosis similar to that seen in KIN is a feature seen in chronic kidney disease in general, the role of DNA damage and repair in other forms of kidney disease may prove to have wider applicability. Indeed, future research could explore whether these findings may be relevant to many diseases in which fibrosis is a hallmark.

*Zhou W, Otto EA, Cluckey A, et al. FAN1 mutations cause karyomegalic interstitial nephritis, linking chronic kidney failure to defective DNA damage repair. Nat Genet 44: 910-915, 2012.*

## KIDNEY DAMAGE: NEW INSIGHTS INTO INITIATION, NEW TARGETS FOR THERAPY

*Multiple recent studies have provided important insights into the origin of scar tissue that is seen in some forms of kidney disease.*

"Fibrosis"—the term that describes the deposition of large amounts of collagen-rich connective tissue that can lead to organ damage—is seen in many conditions related to inflammation and, unchecked, can diminish the ability of an organ to perform its normal functions. In the kidney, fibrosis is a common final pathway for many diseases. It may arise as the result of a brief, severe injury to the kidney—causing acute kidney failure—or from a slowly-progressing, chronic condition. Extensive kidney fibrosis, and the scar tissue that can sometimes arise, can impair the removal of toxins and excess fluid from the blood, cause irreversible organ damage and, in severe cases, lead to kidney failure. New reports shed more light on the origins of kidney fibrosis and identify multiple potential new targets for therapy.

One study focused on molecular regulators of gene expression (the extent to which gene functioning is on or off), and how these regulatory factors might influence the deposition of fibrous tissue following kidney injury. In this study, researchers examined two different mouse models of kidney fibrosis, and sought to identify regulators of gene expression that were elevated in the presence of scarring. The researchers focused on one molecule, microRNA 21 (miR-21) that was found to be highly elevated in two mouse models of kidney disease soon after injury but before fibrosis appeared. This molecule is also found in humans with kidney injury. Mice engineered to lack the miR-21 gene showed diminished fibrosis in response to kidney injury; similar results were observed in normal mice that had been treated with an inhibitor of miR-21. This molecule represents a potential target for antifibrotic therapies in kidney disease.

Another research group identified a cell surface protein, activin-like kinase 3 (Alk3), that is present at elevated levels following kidney injury. Deletion of this protein in certain areas of the kidney leads to increased fibrosis, suggesting that it plays a protective role in the organ. The scientists developed a small, synthetic protein that bound to and activated Alk3. This agent suppressed inflammation and reversed established fibrosis in five different mouse models of kidney disease. Molecules such as this synthetic protein may be able to treat, and possibly reverse, kidney fibrosis.

Two other studies investigated the role of various cell types within the kidney, and tried to identify the source of the collagen-producing cells that can lead to fibrosis. One focused on pericytes, a type of stem cell that is usually associated with blood vessels, in kidney injury and fibrosis. Previous research indicated that kidney fibrosis appears to arise through a pathway involving cells derived from pericytes. The current study found that kidney pericytes increased their levels of the enzyme ADAMTS1, which plays a role in remodeling the tissue surrounding kidney cells, and downregulated an inhibitor of this enzyme, TIMP3, following kidney injury. Mice engineered to lack TIMP3 were more susceptible to kidney injury-induced fibrosis. Together, these results suggest central roles for regulators of enzymes that can modify networks of blood vessels in the kidney following injury.

In a second project focused on the role of a particular type of kidney cell, scientists used a new mouse model to study acute kidney injury and fibrosis. This study involved selective injury to the proximal convoluted tubules, which are part of the nephron, the basic structural and functional unit of the kidney. These tubules resorb about two-thirds of the fluid generated by the glomeruli, the filtering units within the kidney's nephrons. After inducing a one-time injury in a specific region of these tubules, the scientists observed the proliferation of tubular cells and the appearance of inflammatory cells. Following this single injury, the kidney recovered completely. However, when the researchers induced three injuries at one-week intervals, they observed diminished cellular repair, with resultant

blood vessel damage and fibrotic damage to both the kidney tubules and the glomeruli. This study shows that repeated injuries, even to only a portion of the nephron, can lead to more widespread kidney damage, similar to that associated with chronic kidney disease.

Researching another form of kidney disease, a team of scientists used computational and systems biology approaches to examine signaling molecules that regulate gene expression in a mouse model of HIV-associated kidney disease. They identified the protein HIPK2 as a key regulator of kidney fibrosis. Levels of this protein were found to be elevated in both the mouse model and in patients with various forms of kidney disease. Deletion of the gene encoding HIPK2 in the mouse model improved kidney function and reduced the severity of fibrosis. HIPK2 may be a potential target for novel therapies to address kidney fibrosis.

These five studies illuminate the complex system of regulation surrounding kidney fibrosis following injury, and identify multiple potential targets for further strategies aimed at preventing and possibly reversing kidney fibrosis, thereby preserving kidney function. Understanding the cellular and molecular mediators of kidney fibrosis is a high priority for scientists studying kidney disease. The identification of the factors that play a key role in this process might identify new targets for treatment aimed at preventing or reversing fibrosis. Furthermore, a better understanding of fibrosis in general could yield insights into how this process unfolds in other tissues and organs, potentially opening up new avenues to therapy for a range of diseases.

*Chau BN, Xin C, Hartner J, et al. MicroRNA-21 Promotes fibrosis of the kidney by silencing metabolic pathways. Sci Transl Med 4: 121ra18, 2012.*

*Grgic I, Campanholle G, Bijol V, et al. Targeted proximal tubule injury triggers interstitial fibrosis and glomerulosclerosis. Kidney Int 82: 172-183, 2012.*

*Jin Y, Ratnam K, Chuang PY, et al. A systems approach identifies HIPK2 as a key regulator of kidney fibrosis. Nat Med 18: 580-588, 2012.*

*Schrimpf C, Xin C, Campanholle G, et al. Pericyte TIMP3 and ADAMTS1 modulate vascular stability after kidney injury. J Am Soc Nephrol 23: 868-883, 2012.*

*Sugimoto H, LeBleu VS, Bosukonda D, et al. Activin-like kinase 3 is important for kidney regeneration and reversal of fibrosis. Nat Med 18: 396-404, 2012.*

## INSIGHTS INTO INTERSTITIAL CYSTITIS/ PAINFUL BLADDER SYNDROME

**Sex-specific Differences in Pelvic Pain of Interstitial Cystitis/Painful Bladder Syndrome:** Researchers studying interstitial cystitis/painful bladder syndrome (IC/PBS) in a rodent model have found evidence that females experience greater pelvic pain than males, but that this disparity does not correspond to estrogen levels or differences in bladder injury. The majority of IC/PBS patients are women, and some evidence has suggested a role for the hormone estrogen, the primary female sex hormone, in IC/PBS pain symptoms. Researchers investigated this hypothesis in a specific mouse model of IC/PBS. In this model, irritated nerves release a chemical that activates inflammatory cells in the bladder. These inflammatory cells, called mast cells, release histamine and other chemicals that inflame the bladder lining and cause pain. This "neurogenic cystitis" model recapitulates the pelvic pain seen in human patients and is also thought to be one possible pathway for how human IC/PBS develops. Using both female and male mice, the researchers induced nerve irritation with a viral infection and then compared female and male mice for potential sex-specific differences in pelvic pain, and also examined the effects of estrogen levels and genetic background. They found that while one genetic strain of mice experienced more pain than another, female mice from either strain experienced greater pelvic pain than male mice. However, when the scientists ablated estrogen production in some of the female mice prior to

the viral infection, they found no significant differences in pelvic pain between those that had normal levels of estrogen and those that did not. Female and male mice with neurogenic cystitis also sustained similar levels of bladder inflammation and injury, making this a less likely explanation for differences in pelvic pain. These findings suggest that, in this rodent model of neurogenic cystitis, sex differences in pelvic pain exist but are not dictated by estrogen; genetic differences play a role in determining susceptibility to pelvic pain; and the two may be related. Further study of sex differences and the role of genetics in pelvic pain could have important implications for understanding IC/PBS pain in people.

*Rudick, CN, Pavlov, VI, Chen, MC, and Klumpp, DJ. Gender specific pelvic pain severity in neurogenic cystitis. J Urol 187: 715-724, 2012.*

**Clinical Trial Shows Benefit of Specialized Physical Therapy Regimen for Women with Interstitial Cystitis/Painful Bladder Syndrome:** Results from a recent clinical trial suggest that a physical therapy regimen targeting muscle and connective tissue in the pelvic floor, hip, and abdominal areas could help improve symptoms in women with interstitial cystitis/painful bladder syndrome (IC/PBS). In addition to symptoms of pelvic pain, urinary frequency, and/or urinary urgency, many women diagnosed with IC/PBS exhibit tenderness and tension in the muscle and connective tissues surrounding the pelvic area. Previously, a pilot study comparing specialized pelvic floor myofascial physical therapy (MPT) to non-specific, whole-body therapeutic massage among women and men with IC/PBS (in women) or chronic prostatitis/chronic pelvic pain syndrome (in men) had indicated that pelvic MPT might be beneficial specifically for women with IC/PBS. Building on that study, researchers recruited 81 women with IC/PBS of less than 3 years duration for a clinical trial to determine the benefit of pelvic MPT as compared to whole-body therapeutic massage. Participants were randomly assigned to receive up to 10 one-hour sessions of either treatment from a trained physical therapist over the course of 12 weeks. They were then asked to assess overall symptom improvement. Participants were also asked to rate outcomes for specific symptoms and issues related to their condition. The researchers found that, while both groups reported similar improvements in bladder pain, urinary urgency and frequency, and quality of life, 59 percent of the women in the pelvic MPT group reported that their overall symptoms had moderately or markedly improved compared to when they began treatment, versus only 26 percent in the whole-body therapeutic massage group. Neither group reported a serious adverse event during treatment. With these encouraging results in hand, researchers can now pursue questions such as the durability of treatment effects and which patients are most likely to benefit from treatment, as well as other questions that can help determine whether pelvic MPT could become a standard clinical treatment for women with IC/PBS.

*FitzGerald MP, Payne CK, Lukacz ES, et al. Randomized multicenter clinical trial of myofascial physical therapy in women with interstitial cystitis/painful bladder syndrome and pelvic floor tenderness. J Urol 187: 2113-2118, 2012.*

## TREATMENT FOR URINARY INCONTINENCE IN WOMEN

**Specialized Bladder Tests Before Urinary Incontinence Surgery in Women May Be Unnecessary:** Results from a recent clinical trial suggest that invasive and costly tests commonly performed in women before surgery for stress urinary incontinence (SUI) may not be necessary in many cases. Millions of American women suffer from SUI, in which urine leaks from the bladder through the urethra during a physical stress, such as coughing, laughing, sneezing, or exercise. Treatment for SUI includes surgical procedures to support and compress the urethra to stop urine from leaking. Prior to surgery, many women not only receive an office evaluation to diagnose their incontinence, but also undergo specialized bladder function tests called urodynamic studies. These tests help assess how well the bladder, urethra, and muscles that support and compress the urethra work together to store and release urine. Similar to other office bladder procedures, the urodynamic tests can be uncomfortable or painful, and

can increase risk for urinary tract infections. Although the urodynamic tests were found to refine a doctor's diagnosis, the tests have not been proven to guide decisions about treatments or improve surgical outcomes.

To test whether urodynamic studies influenced the likelihood of treatment success, researchers conducted a study in 630 women who were planning to have surgery for SUI. Participants were women who had SUI that was not complicated by other health factors, such as previous incontinence surgery or pelvic irradiation. Women who also had urge incontinence (urine leakage at the time of a very strong desire to urinate, as opposed to physical stress) were not excluded as long as SUI was their predominant type of incontinence. Women with uncomplicated, predominantly SUI were randomly assigned to receive either (1) both a pre-operative evaluation in a doctor's office and urodynamic tests, or (2) the office evaluation only. One year after the surgical procedure, the researchers assessed treatment success, which was defined as a participant reporting on a questionnaire that her urinary distress had been reduced by 70 percent or more, as well as reporting that her urinary tract condition had improved "much" or "very much." The researchers found that the proportion of women in whom treatment was successful was similar in both groups—76.9 percent versus 77.2 percent in the women who had urodynamic testing and the women who received the office evaluation only, respectively—with no significant differences in quality of life, patient satisfaction, or problems voiding. While urodynamic testing did lead to changes in diagnoses for some of the women, the researchers observed that this did not lead to significant differences in either the selection of surgical treatments or the one-year outcomes between the two groups. These results indicate that, for women with uncomplicated SUI who are receiving care from urologists and gynecologists with advanced training in bladder problems, specialized bladder function tests are not necessary to achieve surgical treatment success—information that women and their physicians can consider in planning treatment.

*Nager CW, Brubaker L, Litman HJ, et al. A randomized trial of urodynamic testing before stress-incontinence surgery. N Engl J Med 366: 1987-1997, 2012.*

## NEW WAYS TO TREAT URINARY TRACT INFECTIONS

**Research Yields Potential New Treatment for Urinary Tract Infections:** Researchers have identified novel orally active compounds that appear to block uropathogenic *E. coli* bacteria from binding to bladder cells, thereby preventing new urinary tract infections (UTIs) and mitigating chronic infections in studies in mice. Infections of the urinary tract are common in women—about one-third of all women in the United States are diagnosed with a UTI by the time they reach 24 years of age—and many women experience repeated UTIs. Most UTIs are caused by a common type of *E. coli* bacterium. The outside surfaces of these bacteria contain hair-like projections that are tipped with a sugar-binding protein called FimH. FimH facilitates the binding of bacteria to human or mouse proteins containing mannose, a type of sugar, which are found on the surface of epithelial cells lining the bladder wall. Once attached to bladder cells, the bacteria become resistant to being flushed out by urine and can initiate infection. The bacteria form what is called a biofilm, a well-organized community that adheres to a surface. Bacteria can also invade bladder cells and establish an intracellular reservoir, thus producing a chronic infection. While antibiotic treatments are available, chronic and recurrent UTIs in women have become more challenging due to antibiotic resistance. To circumvent drug-resistant *E. coli*, there is an urgent need for new therapeutics to treat and prevent UTIs.

Because FimH is essential for successful infection by UTI-causing *E. coli*, scientists focused on finding a way to interfere with FimH binding to mannose as a possible new therapeutic strategy. First, they developed a panel of investigational compounds derived from mannose, called mannosides, and tested the ability of these mannose derivatives to block bacterial growth in a laboratory assay designed to mimic a biofilm. They then selected the most promising mannoside compound to evaluate its efficacy in treating and preventing UTI in a mouse model. Four mannoside compounds that were tested blocked biofilm growth when added at an initial stage of biofilm development or were capable of disrupting an established biofilm. The most potent

mannoside was shown not only to reduce bacterial levels in bladders of mice with chronic UTIs, but also to do so more effectively than standard antibiotic treatment. Moreover, when administered prior to exposure to bacteria, the mannoside prevented new UTIs in mice. Like standard antibiotic treatment for UTIs, the mannoside was active when administered orally. Building on these results, the researchers were also able to generate additional, further optimized mannoside compounds as a starting point for new tests.

This promising finding of an alternative approach to UTI treatment emerges from a long-term research investment to understand the virulence and life cycle of uropathogenic *E. coli*, the primary culprit in UTIs in women. Future studies will continue to develop more potent mannosides and to assess these compounds for toxicity prior to their potential testing in humans.

*Cusumano CK, Pinkner JS, Han Z, et al. Treatment and prevention of urinary tract infection with orally active FimH inhibitors. Sci Transl Med 3: 109ra115, 2011.*

## PREVENTION OF BENIGN PROSTATIC HYPERPLASIA IN MEN

**Drug Therapy To Prevent Benign Prostatic Hyperplasia:** The drug finasteride, which inhibits the metabolism of the male sex hormone testosterone and which has been shown to be effective in relieving the symptoms of benign prostatic hyperplasia (BPH), can also reduce the likelihood that otherwise healthy men will develop this condition. While the symptoms of BPH vary, the most common ones involve changes or problems with urination, such as a hesitant, interrupted, or weak stream; urgency and leaking or dribbling; and more frequent urination, especially at night.

Researchers examined patient data from over 9,000 men who were enrolled in the Prostate Cancer Prevention Trial, which collected information on men with BPH and related lower urinary tract symptoms, such as frequent urination, inability to urinate, and urinary tract infections. The average age of the men was 62 years. This retrospective analysis found that men who had received finasteride, a drug that blocks the conversion of testosterone to a more potent metabolite, had a 40 percent lower rate of BPH development than men who did not. The effect of finasteride did not vary significantly by age, race, diabetes, physical activity, or smoking, suggesting that these results could be applicable to a larger population. BPH rarely causes symptoms before age 40, but more than half of men in their sixties and as many as 90 percent in their seventies and eighties have some symptoms of BPH.

For many years, surgery was the only viable treatment option for BPH. In 2003, the NIDDK-supported Medical Therapy of Prostatic Symptoms (MTOPS) clinical trial conclusively demonstrated that combination therapy consisting of an α blocker, which relaxes smooth muscle, and finasteride was more effective than either drug alone in relieving the symptoms of BPH. The current study complements these findings, suggesting that finasteride may be an effective preventative therapy in men without overt symptoms of BPH.

*Parsons JK, Schenk JM, Arnold KB, et al. Finasteride reduces the risk of incident clinical benign prostatic hyperplasia. Eur Urol 62: 234-241, 2012.*

## IRON: A DELICATE BALANCE

**The Double-edged Sword of Hepcidin:** Approaches to modulate levels of hepcidin may provide benefit to people with either iron overload or iron deficiency. Hepcidin, a peptide hormone produced by the liver, is the master regulator of iron balance in humans and other mammals. Hepcidin inhibits transport of iron from cells by binding to the iron channel, ferroportin, which reduces dietary iron absorption and limits release of iron from cells that store iron, such as macrophages. Insufficient levels of hepcidin cause or contribute to iron overload in β-thalassemia and in hereditary hemochromatosis, while excess levels of hepcidin lead to a decline in blood iron levels, as occurs in the anemia of chronic inflammation. Thus, strategies that increase or decrease the effective level of hepcidin could help treat these diseases.

Researchers recently reported the design of smaller forms of hepcidin and tested the ability of these compounds to mimic hepcidin activity and also treat iron overload in mice. These "mini-hepcidins" contain the segment of the hepcidin protein that interacts with ferroportin, and were found to be resistant to degradation by enzymes in the blood. After injection or oral administration to mice, these "mini-hepcidins" were found to lower serum iron levels as effectively as full-length hepcidin and also to lower liver iron levels significantly in an animal model of iron overload. "Mini-hepcidins" offer certain advantages over full-length hepcidin in that they are less expensive to produce, are more stable, and can be administered orally.

In a separate study, scientists assessed the ability of two small compounds (LDN-193189 and HJV.Fc) to block the production of hepcidin in an animal model of anemia of chronic inflammation. Previous research has demonstrated that both LDN-193189 and HJV.Fc block the action of a protein that signals the hepcidin gene to be expressed. By blocking this protein, called bone morphogenetic protein (BMP), the production of hepcidin was effectively blocked. This study provided compelling evidence that both agents reduced hepcidin levels by blocking BMP, allowing iron to become more available for red blood cell production, thereby ameliorating anemia of chronic inflammation in an experimental animal model.

Past investments in basic science research provided the foundation for these two research studies targeting hepcidin levels. Ongoing studies continue to evaluate these and other promising compounds in order to develop effective treatments for iron-related blood disorders.

*Preza GC, Ruchala P, Pinon R, et al. Minihepcidins are rationally designed small peptides that mimic hepcidin activity in mice and may be useful for the treatment of iron overload. J Clin Invest 121: 4880-4888, 2011.*

*Theurl I, Schroll A, Sonnweber T, et al. Pharmacologic inhibition of hepcidin expression reverses anemia of chronic inflammation in rats. Blood 118: 4977-4984, 2011.*

## GENE REGULATION AND BLOOD CELL FORMATION

**Blood Cell Formation and Regeneration:** Scientists have recently determined that two biologic pathways, the bone morphogenetic protein (BMP) and Wnt signaling pathways play a dynamic role in blood cell (hematopoietic) regeneration and maturation (differentiation). Following an insult or injury to the blood system, such as rapid blood loss due to a serious injury, the regenerative process stimulates rapid expansion of hematopoietic stem cells followed by differentiation of these cells into red blood cells and other major blood cell types. Both BMP and Wnt signaling pathways contribute to the initial formation of the hematopoietic system during development, but it was not known whether these pathways are also involved in hematopoietic regeneration and differentiation after injury during adulthood.

To evaluate whether BMP and/or Wnt signaling pathways contribute to blood cell regeneration, scientists performed experiments on zebrafish, a model organism. They subjected adult zebrafish to a sub-lethal dose of radiation to destroy their existing blood cells. They then looked for signs of blood cell regeneration by characterizing blood cell populations from zebrafish kidney marrow, which is considered the organ responsible for production of all major blood cell types, analogous to the mammalian bone marrow. The regeneration of blood cells following irradiation of the fish was shown to depend on the activation of both the BMP and Wnt signaling pathways.

The researchers next gained insight into how these signaling pathways promote regeneration of different types of blood cells. Drawing upon previous findings that the BMP and Wnt pathways act by turning genes on or off, the scientists sought to identify other factors involved in this process. Two gene-regulating factors, SMAD1, a member of the BMP signaling family, and TCF7L2, a member of the Wnt signaling family, were found to be associated with genes that play a role in the production of blood cells. Furthermore, when the researchers examined red blood cells grown in the laboratory, these factors were found to be

bound to DNA in close proximity to known "master regulators" GATA1 and GATA2, which are important for directing gene regulation in a particular progenitor cell so that it becomes a red blood cell. Interestingly and importantly, in a white blood cell line, SMAD1 and TCF7L2 were not found to be associated with red blood cell genes in the zebrafish DNA, but rather were associated with white blood cell genes. In these cells, SMAD1 and TCF7L2 were both in close proximity to a different master regulator, C/EBPα—important for directing a particular progenitor cell to become a white blood cell.

This study identifies signaling pathways and factors associated with different master regulators of genes, which direct the regeneration and differentiation of distinct blood cell types in adults following injury.

*Trompouki E, Bowman TV, Lawton LN, et al. Lineage regulators direct BMP and Wnt pathways to cell-specific programs during differentiation and regeneration. Cell 147: 577-589, 2011.*

## NEW APPROACHES TO TREATING ANEMIA

**Nutritional Supplements Can Mitigate the Severity of Two Serious Forms of Anemia in an Experimental Model:** Diamond-Blackfan anemia (DBA), an inherited form of bone marrow failure, and myelodysplastic syndrome arising from a deletion of a portion of chromosome 5 [del(5q) MDS], an acquired disease, are both characterized by anemia, or insufficient levels of red blood cells. As these cells carry oxygen from the lungs to all of the body's organs and tissues, deficient numbers of red blood cells can cause a wide range of serious medical issues. A new study, based on experiments in an animal model and with cultured human cells, suggests that the anemia associated with these disorders can be alleviated with an amino acid supplement.

Previous studies have indicated that DBA and del(5q) MDS are caused by an insufficient level of one or more of the protein components of ribosomes. Ribosomes are complex structures of proteins and ribonucleic acids that perform the final step of translating the DNA "blueprint" into proteins that carry out the functions of cells and tissues. Mutations in nine genes that encode ribosomal proteins have been identified in patients with DBA, accounting for about half of the diagnosed cases.

In the current study, researchers modeled DBA and del(5q) MDS in zebrafish using a targeted approach to reduce the levels of two specific ribosomal proteins that have been implicated in these diseases. They observed that, as in humans, diminished levels of these proteins in zebrafish resulted in severe anemia. When the animals were treated with leucine, there was a significant improvement in the animals' anemia. Similar results were seen in experiments with cultured human blood cells. Leucine is an "essential" amino acid that must be part of the diet because it cannot be synthesized in animals and humans. Leucine has previously been shown to enhance ribosome function in cells, potentially by activating a biologic pathway (known as the mTOR pathway) that integrates multiple signals inside the cell and plays a role in protein synthesis. In this study, the researchers showed that leucine activates this pathway in blood cells, a result that may explain how leucine improved anemia in the zebrafish.

The only true cure for DBA is a bone marrow transplant, which is a very limited treatment approach because suitable bone marrow donors are rarely available and serious risks are associated with the procedure. The novel findings described in this study show that simple nutritional supplementation may reverse many of the manifestations of DBA and del(5q) MDS and provide a rationale for future studies that will explore the potential effectiveness and safety of nutritional leucine supplements to treat patients with these rare disorders.

*Payne EM, Virgilio M, Narla A, et al. L-leucine improves the anemia and developmental defects associated with Diamond-Blackfan anemia and del(5q) MDS by activating the mTOR pathway. Blood 120: 2214-2224, 2012.*

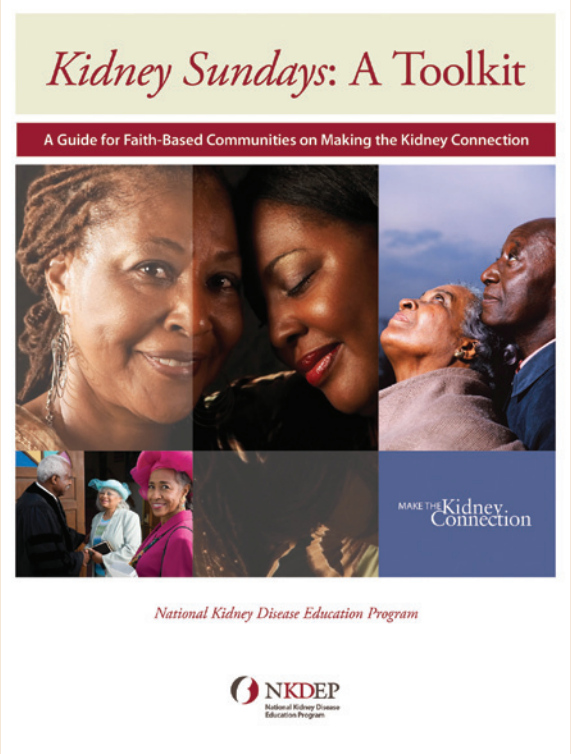
# National Kidney Disease Education Program's *Kidney Sundays* Initiative

## *Raising Awarenesss of Kidney Disease Risk Factors Among African Americans*

According to data from the National Health and Nutrition Examination Survey (NHANES), 16 percent of African Americans may have chronic kidney disease (CKD). African Americans between the ages of 30 and 39 are 11 times more likely than Caucasians to develop hypertension-related kidney failure. Additionally, the same age range of African Americans is almost four times more likely than Caucasians to develop diabetes-related kidney failure.[1] While African Americans made up just 13 percent of the United States population in 2009, they accounted for 32 percent of new cases of kidney failure.[2] In addition to diabetes and hypertension, heart disease and a family history of kidney failure also increase kidney disease risk.

To help African Americans learn about kidney disease and what steps they can take if they have risk factors, the National Kidney Disease Education Program (NKDEP) conducts community outreach and education programs. In recognition of National Kidney Month in March 2012, NKDEP collaborated with the American Diabetes Association's *Live Empowered* program; the National Coalition of Pastors' Spouses (NCPS); and Chi Eta Phi Sorority, Incorporated to kick off the first nationwide *Kidney Sundays* event.

"We want people to take a more active role in protecting their kidneys—and that means if they have diabetes or high blood pressure, making lifestyle changes to manage these diseases," said Griffin P. Rodgers, M.D., M.A.C.P., Director, National Institute of Diabetes and Digestive and Kidney Diseases. "*Kidney Sundays* provided us an opportunity to make sure people know how important staying healthy is and what they can do if they are at risk for kidney disease."




On March 25, 2012, more than 350 African American faith communities from Baltimore to St. Louis, and from Dallas to Los Angeles, used NKDEP's newly revised *Kidney Sundays* Toolkit to help their members learn about the connection between diabetes, high blood pressure, and kidney disease. Thirty-nine of the participating congregations also conducted more than 1,500 blood pressure screenings with Chi Eta Phi nurses on site. The nurses shared kidney health information and encouraged those at risk to have their kidneys tested. In addition, more than 300 NCPS members also shared kidney health information in their churches.

"We still have lots of work ahead of us in terms of educating people on the serious effects of diabetes, obesity, high blood pressure, and eating habits, and how each relates to kidney health," said NCPS President Vivian Berryhill, of New Philadelphia Baptist Church in Memphis, Tennessee. "Efforts such as *Kidney Sundays* are a great start to help… get the conversations started."

The *Kidney Sundays* partnership with participating faith organizations helped NKDEP reach an estimated 280,000 individuals through distribution of 50,000 educational pieces, including *Kidney Sundays* Toolkits, brochures, and information cards.

In addition to the faith community engagement, NKDEP also conducted a national media campaign featuring Dr. Rodgers. The campaign included a radio tour with segments on African American stations and shows including the *Tom Joyner Morning Show* and a partnership with *BlackDoctor.org* to host a kidney health chat with Dr. Rodgers on its Facebook page. These efforts, along with a robust social media conversation on the NKDEP *Make the Kidney Connection* Facebook page, garnered 3,667 visitors and 458 material downloads on the NKDEP web-site, and reached more than 60,000 individuals through eNewsletters distributed by NKDEP, the NIDDK and its programs, and partner organizations from January through March 2012.

*More information about the NIDDK's National Kidney Disease Education Program can be found at http://nkdep.nih.gov. To learn more about Kidney Sundays, visit http://nkdep.nih.gov/kidneysundays*

[1] *U.S. Renal Data System, USRDS 2012 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2012.*

[2] *U.S. Renal Data System, USRDS 2010 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2010.*

# STORY OF DISCOVERY

## *Small Molecule Stabilizers as a Potential Treatment for Transthyretin Familial Amyloid Polyneuropathy*

Proteins are important building blocks for all body parts, including muscles, bones, hair, and nails, and perform vital biologic functions. Proteins circulate throughout the body in the blood and, when functioning properly, are beneficial. Occasionally, cells produce abnormal proteins that can misfold, form fibrillar deposits or aggregates and cause disease; the exact cause of fibril formation is unknown. When these deposits of abnormal proteins were first discovered, they were called amyloid, and the disease process was called amyloidosis. A common feature is that the deposits share a "β-pleated sheet" structural conformation with characteristic properties detectable by certain color dyes. Diagnosis of the various forms of amyloid disease is confirmed by tissue biopsy. In systemic amyloidosis, proteins produced in one part of the body travel to a different location where they become insoluble and form fibrillar deposits that impair organ function. One such form of systemic amyloid disease is transthyretin (TTR) amyloidosis. Past investments in basic science research have provided the foundation for an exciting small molecule approach to inhibit TTR amyloidosis.

**The Blood Transport Protein Transthyretin in Health and Disease**

TTR is a blood transport protein for the hormone thyroxine and the vitamin A-retinol binding protein complex. TTR has two binding sites for thyroxine but only a small percentage, perhaps less than 1 percent, of TTR in blood has thyroxine bound to it. The liver is the main site of TTR synthesis in the body, but TTR is also made in the retina and pancreas. Both mutant and normal forms of TTR can give rise to amyloid deposits. TTR consists of four identical subunits; scientists refer to the assembled subunits as a tetramer. Dissociation of the tetramer is the rate-limiting step for amyloid fibril formation. Any one of nearly 100 different mutations in the gene encoding TTR can cause amyloidosis. Disease-associated mutations destabilize the tetrameric structure and some increase the rate of tetramer dissociation. Specific clinical syndromes associated with mutated TTR are familial amyloid polyneuropathy (FAP) and familial amyloid cardiomyopathy. Senile systemic amyloidosis is a disorder that occurs in very elderly individuals (mostly men) in which amyloid fibrils formed from the normal TTR protein are deposited primarily in the heart, but also in the gut and in the carpal tunnel space of the wrist.

**What is Transthyretin Familial Amyloid Polyneuropathy?**

TTR FAP is a rare, progressive, and ultimately fatal hereditary neurodegenerative disease that affects the nerves and often the heart and kidneys as well. Symptoms include sensory loss, erectile dysfunction, alternating diarrhea and constipation, urinary incontinence, urinary retention, and delayed gastric emptying. TTR FAP is inherited in an autosomal dominant manner. The phrase "autosomal dominant" means that if one parent has the disease, there is a 50 percent chance that the disease gene will pass to a child. A mutation in TTR called "V30M" is the most common cause of FAP. Currently, there are no

# STORY OF DISCOVERY

FDA-approved drugs to treat this rare but serious disease. Liver transplantation is a treatment option, as replacing a liver producing mutant TTR with a liver synthesizing normal TTR can slow, if not halt, disease progression. However, this approach has its limitations, which include matched donor availability, surgery, and a need for long-term immunosuppressive steroid treatment after the transplant.

**Development and Characterization of TTR Small Molecule Stabilizers**

*NIDDK-supported scientist Dr. Jeffery W. Kelly of the Scripps Research Institute and his collaborators have conducted seminal research studies to design and characterize small molecules that stabilize the native TTR tetrameric structure. Highlights of some of these pre-clinical and clinical studies are presented here.*

In the laboratory, the stability of TTR's tetrameric structure can be assessed by placing it in a chemical known to disrupt or "denature" protein structures. In 1996, a strategy was developed to design an orally bioavailable small molecule stabilizer that binds to TTR in blood with both high affinity and selectivity, and prevents or significantly slows dissociation of the TTR tetramer. In a proof of principal experiment, thyroxine concentrations slightly higher than necessary to occupy all TTR binding sites stabilized both normal and mutant-containing (*e.g.*, V30M) tetramers from dissociation under conditions of denaturation. Unfortunately, thyroxine cannot be used as a stabilizer of tetramer structure—owing to its hormone activity. However, results of this study did provide evidence that a small molecule having a similar structure to thyroxine without hormonal activity but with specific and selective binding affinity might effectively stabilize normal and mutant TTR. A related research study subsequently showed that occupancy of only one of the two thyroxine binding sites is sufficient to stabilize most TTR tetramers from dissociation under denaturing conditions.

Based on structural similarity to thyroxine, a small molecule called diclofenac was tested for its ability to stabilize TTR under denaturing conditions, and researchers also evaluated a set of 12 similar molecules. These were nonsteroidal anti-inflammatory small molecules (NSAISMs), and in 2002, several, including diclofenac, were shown to stabilize the normal TTR tetramer effectively, but they were less effective at stabilizing mutant TTR tetramers (*e.g.*, V30M). In 2004, diclofenac and several additional NSAISMs were evaluated for their ability to stabilize tetramer structure of the most common disease-associated TTR variants, including V30M. This study demonstrated that the NSAISM diflunisal provided effective stabilization for the majority of the mutant variants and was more effective in this regard than diclofenac. Used as an FDA-approved non-steroidal anti-inflammatory drug for more than 2 decades, diflunisal has been commercially available in over 40 countries, including the United States.

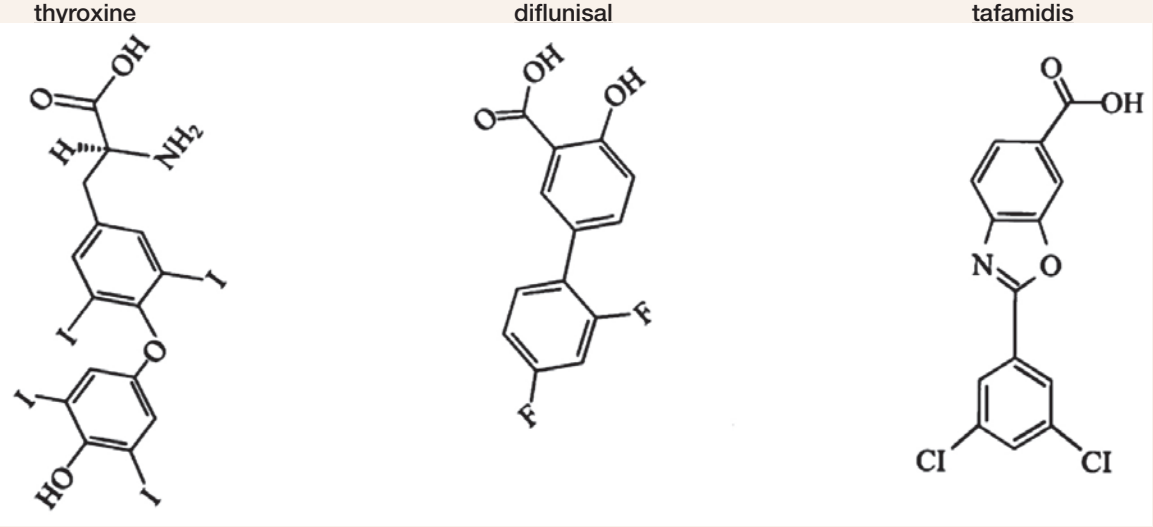
Diflunisal was evaluated further for its ability to selectively bind and stabilize 1) FAP variant TTR in blood samples, and 2) TTR when orally administered to healthy volunteers or patients with FAP. When diflunisal was added to the blood of FAP patients at potentially therapeutic concentrations, mutant V30M TTR tetramers in the blood were significantly stabilized under denaturing conditions, more so than the TTR of healthy volunteers. Already FDA-approved for mild to moderate pain, fever, and inflammation, orally administered diflunisal at 250 mg twice daily for 7 days was shown to stabilize TTR in blood samples obtained from a small pilot study of patients with FAP. Moreover, a second pilot study of orally administered diflunisal at

# STORY OF DISCOVERY

250 mg or 500 mg twice daily for 7 days showed that the drug occupied at least 1 of the 2 thyroxine binding sites and stabilized TTR tetramer structures from healthy volunteers. Published in 2006, the results of these two pilot studies suggested that diflunisal might be an effective small molecule therapeutic for treating TTR amyloidosis.

In 2003, a series of compounds called benzoxazoles, which also have a structure similar to thyroxine, were synthesized, and 11 of 28 were shown to effectively stabilize normal TTR tetramers and to prevent amyloid fibril formation under denaturing conditions. One of these analogs, tafamidis, exhibited particularly favorable binding selectivity and stabilization of the normal TTR tetramer. Because of these findings, tafamidis was selected for additional pre-clinical and clinical research studies. As reported in 2012, tafamidis was found to effectively stabilize tetramers of the most clinically significant mutant form of TTR (V30M) associated with FAP, such that it behaves like normal TTR under denaturing conditions. Occupancy of one of the thyroxine binding sites by tafamidis stabilized 67 percent of normal TTR tetramers from dissociation under denaturing conditions. Increasing tafamidis concentrations such that both thyroxine binding sites were occupied stabilized 97 percent of the TTR tetramers. Consistent with previous results, tafamidis was also shown to selectively bind and stabilize TTR in human blood. When added to blood from patients with FAP at concentrations sufficient to occupy one or both of the thryoxine binding sites, tafamidis was found to significantly stabilize the V30M TTR under denaturing conditions. Furthermore, tafamidis was shown to stabilize a broad range of other pathogenic TTR variants in blood; these TTR variants contained mutations called Y69H, F64S, I84S, L111M, or V122I.

**Thyroxine and Candidate Small Molecule Stabilizers**



# STORY OF DISCOVERY

**Clinical Trial Assessments of Diflunisal and Tafamidis**

These two small molecule stabilizers have now been studied in clinical trials to test their safety and efficacy. Boston University has sponsored a placebo-controlled multicenter phase III clinical trial testing the efficacy of diflunisal for the treatment of FAP, and results are expected to be reported in 2013. This trial, with a target enrollment of 140 participants, has compared 250 mg diflunisal taken orally twice daily with a placebo. Results of a separate clinical trial evaluating tafamidis were reported by the Scripps Research Institute in July 2009. This 18-month phase II/III clinical trial, conducted by a pharmaceutical company co-founded by Dr. Kelly, showed that tafamidis slowed the progression of FAP in patients with the V30M TTR mutation. Further testing is now ongoing to achieve U.S. Food and Drug Administration approval of tafamidis for the treatment of TTR FAP.

**NIDDK-supported Translational Research**

The translation of scientific knowledge and technology into improvements in the practice of medicine is central to the missions of the NIH and the NIDDK. As this story illustrates, the clinical understanding of the pathological underpinnings of TTR amyloidosis spurred NIDDK support of basic science research that led to the development of small molecule stabilizers that effectively decrease TTR tetrameric dissociation and amyloid fibril formation. Once identified and characterized, these small molecules were well positioned to attract academic and industry interest in conducting clinical trials of these potential therapeutics. The NIDDK continues to be committed to supporting innovative strategies for improving the health of patients with FAP and many other diseases.

# SCIENTIFIC PRESENTATION

## From Genes to Therapies: Platelets at the Center of the Universe

*Dr. Kenneth Kaushansky*

*Dr. Kenneth Kaushansky is Senior Vice President, Health Sciences and Dean, School of Medicine at Stony Brook University. A physician-scientist and leading hematologist, Dr. Kaushansky has conducted groundbreaking research on the molecular biology of blood cell production. His team has discovered several of the genes important in the growth and development of blood cells, including thrombopoietin, a key regulator of stem cell and platelet production. Dr. Kaushansky's laboratory work has led to several significant discoveries, for which he has received the Dameshek Award from the American Society of Hematology, awarded annually to the scientist who has made seminal contributions into the field of hematological disorders, and the Outstanding Investigator Award from the American Society for Medical Research, the most prestigious award of the Society.*

*Dr. Kaushansky earned his B.S. and M.D. degrees from the University of California, Los Angeles, and completed his Internal Medicine Internship, Residency and Chief Medical Residency, and Fellowship in Hematology at the University of Washington. He joined the faculty at the University of Washington as an Assistant Professor in 1987, was promoted to Associate Professor in 1991 and to Professor in 1995. Following his service as Hematology Section Chief at the University of Washington Medical Center, Dr. Kaushansky was named Helen M. Ranney Professor and Chair of the Department of Medicine at University of California, San Diego, in February 2002. In July 2010, Dr. Kaushansky moved to Stony Brook University.*

*Dr. Kaushansky has been an NIH-supported researcher for the past 30 years, including research support from the NIDDK for 27 years. At the September 2012 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, Dr. Kaushansky gave a presentation on the role of the platelets in bleeding and clot formation.*

Platelets are cell fragments that help blood clot. Too few platelets can lead to spontaneous or uncontrolled bleeding. Too many platelets can trigger a heart attack, stroke, or other arterial thromboses. As such, regulation of the numbers and function of platelets is vital for health—the yin and yang of platelets.

**How is Platelet Production Regulated?**

Dr. Kaushansky recounted that, for many years, the primary regulator of blood platelet production remained elusive. Then, in the late 1980s and early1990s, a French research group reported that a gene, *v-mpl*, from the murine (mouse) myeloproliferative leukemia virus (MPLV) had the ability to transform, or "immortalize," a variety of early stage blood cells. The protein encoded by *v-mpl* was shown to be very similar (homologous) to members of a family of cell-surface receptors, including those for growth hormone and erythropoietin, the regulator of red blood cell production. This finding prompted speculation that a non-viral, mammalian form of this receptor may exist and transmit signals for a critical hormone. In 1992 and 1993, two research groups described the identification of human and mouse

# SCIENTIFIC PRESENTATION

*c-mpl*—the homolog of *v-mpl*—and confirmed that the *c-mpl* gene encoded for a new protein belonging to the blood cell (hematopoietic) growth factor family of receptors. Knowledge of the c-Mpl protein quickly led to the discovery of the protein that binds to this receptor. Dr. Kaushansky's research group reported the isolation of the protein—thrombopoietin—that specifically interacts with the c-Mpl receptor. To confirm its function in blood cell production, Dr. Kaushansky and his colleagues treated mice for several days with thrombopoietin that had been synthesized in the laboratory and showed that platelet numbers increased dramatically compared to untreated (control) animals. These reports, and subsequent in-depth molecular and cellular studies, helped to delineate the physiological control of platelet production.

All blood cell types are derived from a population of self-renewing hematopoietic stem cells (HSCs) that first appear during embryonic development. In the cell lineage leading to platelet production, the HSC gives rise to progressively committed progenitor cells that each ultimately yields thousands of platelets. Specifically, HSCs become multipotent progenitor cells, which then become progenitors for specific types of blood cells, such as platelets. In the lineage of platelet development, progenitor cells give rise to large cells called megakaryocytes, first in immature and then mature forms. In a step that is unique in biology, individual megakaryocytes fragment into 1,000 to 3,000 platelets. Prior to fragmentation, the megakaryocyte undergoes a process that results in a many-fold geometric increase of chromosome numbers in a single "polyploid" nucleus. In humans, a cell normally contains 23 chromosome pairs, or a total of 46 chromosomes, in its nucleus. Mature megakaryocytes usually contain 8, 16, 32, or 64 times this normal number of chromosome pairs.

So how does the megakaryocyte become polyploid? Dr. Kaushansky explained that the megakaryocyte undergoes a process called endomitosis—the DNA is repeatedly replicated in the absence of cell division (mitosis); the cell does not divide into two daughter cells as would normally occur during chromosome duplication. This process is not simply the absence of mitosis but rather a culmination of several cycles of aborted mitoses. Dr. Kaushansky and his team systematically evaluated a handful of proteins which could be responsible for endomitosis. By comparing how these proteins behaved in normal cells undergoing cell division compared with the unique process taking place within the megakaryocyte, the evidence pointed to a protein called RhoA. The exact role that RhoA is playing in this process is now under investigation.

Although many researchers in the field predicted that thrombopoietin had physiological effects limited to megakaryocytes and platelets, Dr. Kaushansky and his colleagues studied its potential effect on HSCs. HSCs were placed in a less than optimum cell culture broth in the laboratory, and the ability of thrombopoietin and other factors to support cell survival was then evaluated. In the absence of thrombopoietin or other factors, all cells died within a few days. When stem cell factor or IL-3 were added to the cells, 80 to 90 percent of the cells survived up to 7 days. Somewhat surprisingly, thrombopoietin was also found to support the survival of HSCs similarly.

In medical situations where patients have blood cell cancers or are undergoing high-dose chemotherapy, HSCs harvested from related family members or from the patients themselves are commonly used by physicians to infuse into the patients' blood stream in order to repopulate their hematopoietic system. Although these "transplantations" have been used for several decades, the exact mechanisms governing the re-establishment of the hematopoietic system

and blood cell production are not fully understood. Paramount to successful repopulation of the hematopoietic system is the "homing" of HSCs from the blood stream to the bone marrow. It is in the bone marrow that HSCs undergo multiple cell divisions to produce an expanded number of HSCs and to begin to mature into committed progenitor cells.

Dr. Kaushansky's team next sought to determine whether thrombopoietin plays a biologically relevant role in HSC expansion. Bone marrow cells were obtained from either mice genetically altered to no longer produce thrombopoietin or normal mice, and normal HSCs were transplanted into the two types of mice. Five to 8 weeks post-transplantation, the number of HSCs in the transplanted mice was determined; Dr. Kaushansky found approximately 17-fold greater numbers of HSCs in the normal mice than in animals in which thrombopoietin was missing. Clearly, in mice, thrombopoietin and the thrombopoietin receptor are critical for stem cell expansion.

Dr. Kaushansky and his collaborators have also begun mapping how thrombopoietin exerts its influence on cells and tissues. After thrombopoietin binds to its cell surface receptor, c-Mpl, the receptor initiates a cascade of events that results in a cellular response. This process is termed signal transduction; the key intracellular protein involved in thrombopoietin signaling is the protein phosphorylation enzyme, JAK2.

**Thrombopoietin: Clinical Implications**

Approximately 30 to 50 children each year are born with congenital amegakaryocytic thrombocytopenia—born without megakaryocytes and hence very low platelet counts, approximately 5 to 10 percent of normal. By age one, most of these children have developed bone marrow failure and pancytopenia (or low numbers of all blood cells) due to the lack of HSC in their bone marrow. The molecular defect in this rare disease has been determined to be mutations in c-Mpl. Thus, this "accident of nature" proves that human HSCs, and hence all blood cell types, like those of mice, are very much dependent on the presence of thrombopoietin and c-Mpl.

Dr. Kaushansky noted several potential clinical uses of thrombopoietin therapy to increase stem cell and platelet population: hematopoietic recovery following chemotherapy for cancer; thrombocytopenia (inadequate platelet count) associated with HIV; aplastic anemia; myelodysplastic syndrome, a condition in which stem cells are defective and do not mature normally; and immune (antibody-mediated) thrombocytopenia. Another potential use was to improve platelet donor yields. Until now, the only therapy available for patients with thrombocytopenia has been the transfusion of platelets obtained from healthy donors. In one trial, administration of a normal form of thrombopoietin was found to ameliorate chemotherapy-induced severe thrombocytopenia in patients with ovarian cancer and reduced the need for platelet transfusions. In a second trial, a single dose of a modified form of thrombopoietin increased platelet counts from 225,000 to 600,000 in healthy platelet donors, allowing recovery of three times more platelets for transfusion. Unfortunately, multi-dose treatment of the healthy donors with this modified form of thrombopoietin led to the development of neutralizing antibodies to the modified protein that also recognized the donors' own thrombopoietin and caused thrombocytopenia in the donors. Because of this serious side effect, the modified form of thrombopoietin was withdrawn from clinical trials in the United States in 1998.

Hampered by the appearance of neutralizing antibodies to the modified form of thrombopoietin,

researchers have sought to develop small molecules that would mimic the effect of thrombopoietin but without stimulating the production of neutralizing antibodies. Two small molecules—Romiplostim and Eltrombopag—have been developed that bind to different sites on c-Mpl, but nonetheless mimic thrombopoietin signal transduction. In 2008, both Romiplostim and Eltrombopag were shown to be effective and well tolerated in patients with chronic immune thrombocytopenia (ITP) who had failed several therapies for the disease; platelet counts increased within 1 to 2 weeks and were sustained throughout 24 weeks of treatment. Both drugs were then approved for use in patients with refractory ITP.

**Blood Cell Cancers**

Dr. Kaushansky then informed Council members that mutations have been identified in both *thrombopoietin* and *c-mpl* genes in humans. Congenital *thrombopoietin* mutations cause an overproduction of thrombopoietin, leading to familial thrombocythemia (high platelet counts) and are associated with blood clots. Congenital *c-mpl* mutations cause both amegakaryocytic thrombocytopenia (see above) and familial thrombocythemia, due to activating mutations, while acquired activating mutations of *c-mpl* have been found to cause essential thrombocythemia and primary myelofibrosis—two blood cell cancers (myeloproliferative neoplasms) that lead to morbidity and mortality from either bleeding or excessive blood clotting.

The most common mutation that causes myeloproliferative neoplasms is found in the thrombopoietin signaling protein JAK2. In fact, a single acquired mutation in JAK2 is found in up to 97 percent of patients with polycythemia vera (increase in all blood cell types, particularly red blood cells), approximately half of patients with essential thrombocythemia and patients with idiopathic myelofibrosis (increased collagen deposition in bone marrow), but is absent in healthy individuals and patients with other hematological cancers. How the same JAK2 mutation can cause three distinct clinical conditions remains unclear.

To better understand the consequences of mutated JAK2, a colleague of Dr. Kaushansky's, Dr. Radek Skoda, designed a genetically engineered mouse model which allowed the researchers to control mutant JAK2 production in the mice, compared to normal JAK2. This study showed that mice producing lower levels of mutant JAK2 compared to normal protein displayed characteristics of essential thrombocythemia, whereas those with high levels of the mutant protein exhibited characteristics resembling polycythemia vera. Thus, the ratio of mutant JAK2 to normal JAK2 determines the myeloproliferative neoplasm phenotype in this mouse model.

Dr. Kaushansky's research group has recently established additional mouse models to further explore the role of mutant JAK2 in myeloproliferative neoplasms. They engineered different mice to produce mutant JAK2 in certain, but not all, of their cells. One group of mice produced mutant JAK2 in their hematopoietic and endothelial cells (the cells lining the inside of blood vessels); another group produced the mutant protein in their megakaryocytes and platelets; and other groups of mice produced this protein only in their white blood cells, endothelial cells, or bone marrow cells. The mouse line producing mutant JAK2 in hematopoietic and endothelial cells was found to have increased platelets and white cells but not red blood cells. These five mouse lines are currently under investigation to determine whether they have abnormal clotting or bleeding characteristics.

# SCIENTIFIC PRESENTATION

**Future Directions**

Dr. Kaushansky noted that there are many future research challenges for studies of thrombopoietin. These include 1) understanding the molecular mechanisms underlying thrombopoietin's effects on hematopoietic stem cell cycle regulation and cell survival; 2) determining the exact role that RhoA plays in endomitosis; and 3) delineating the molecular mechanisms of thrombopoietin on platelet formation. In addition, research is needed to better understand myeloproliferative neoplasms, including the role played by thrombopoietin and its receptor c-Mpl. Once researchers gain a better understanding of these disorders, they can use this knowledge to design and develop new, more effective therapies.

# PATIENT PROFILE

## Veronica Garcia

*Balancing Life with Interstitial Cystitis/Painful Bladder Syndrome Includes Participating in Research*

Veronica Garcia

Thirty-four-year old Veronica Garcia has been dealing with urinary pain symptoms for most of her life. "Even as a 3- or 4-year old child, I remember it was painful to go," she recalls. Although her doctors suspected chronic urinary tract infections and over the years treated her with antibiotics and palliative measures for pain, no one could give her a specific diagnosis. However, when her symptoms changed about a year or so ago, Veronica and her doctor became concerned, and she received a new workup from a urologist specializing in pain issues. Now diagnosed with interstitial cystitis/painful bladder syndrome (IC/PBS), a chronic urologic pain syndrome for which there is no widely effective treatment or cure, Veronica has enrolled in the NIDDK-supported Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network. This multi-site study is seeking answers about the fundamental causes, progression, and nuances of urologic chronic pelvic pain syndromes that may help people with IC/PBS.

**"Bad Plumbing"**

Veronica, who works full-time as a computer programmer/analyst, is a problem solver who is determined not to let symptoms of IC/PBS get in the way of her life. A wife and mother of two, Veronica bubbles with enthusiasm as she describes how much she enjoys her family and volunteering, especially for activities with her son, Jesse (age 13), and daughter, Isabella (age 10). "Jesse is in football, and Isabella, who is very athletic, is doing cheer(leading)," she says. Both children are also in Scouts, which Veronica thinks is great for skill building and being part of the community. Additionally, Veronica says she is "very crafty" and loves to do holiday decorations and creative projects with her children any chance she gets. According to Veronica, activities like these are "very important," as is her involvement. She also loves to read and share book recommendations with friends—"a great way to keep in touch," she says.

However, Veronica has been dealing with pain and pelvic and abdominal discomfort issues most of her life. "The running joke is that I have 'bad plumbing,'" she says with a laugh. After first noticing pain in

# PATIENT PROFILE

childhood, Veronica's urinary pain worsened as she grew older, peaking in her teens and twenties. She also sometimes had blood in her urine. Sometimes the pain would be so bad that she couldn't sit still, and she would be afraid to go to the bathroom. Tightly fitted clothing wasn't an option. "There was a period of time when I wore overalls all the time," says Veronica. During the worst times, she says, "I sometimes felt like life wasn't fair. Other people could run around and do things without a care, and I was having to make all kinds of life adjustments."

About 6 years ago, Veronica had her first workup with a urologist. While she was glad to find out that she did not have cancer or another life-threatening condition, she still did not have a diagnosis. She says that she was told that, regarding her urinary pain and sensitivity, "some people are just like that." She continued to be prescribed antibiotics, which, Veronica says, sometimes helped, but sometimes didn't—most likely, she thinks, because she occasionally did have an actual urinary tract infection that was causing pain.

Five years later, Veronica says, her symptoms changed. She started experiencing pain in her lower back, near her kidneys, which, she says, was "very scary"; she also felt increased pressure on her bladder. At about the same time, a relative was diagnosed with IC/PBS. As she discussed the symptoms with her relative, Veronica began to think that she might also have IC/PBS. After discussing her new symptoms and her relative's diagnosis with her primary care doctor, she was sent to a different urologist specializing in these issues, who did another workup—and diagnosed Veronica with IC/PBS.

**What Is IC/PBS?**

Interstitial cystitis (IC) is a condition that results in recurring discomfort or pain in the bladder and the surrounding pelvic region. Symptoms vary from person to person and even in the same individual at different times. People may experience mild discomfort, pressure, tenderness, or intense pain in the bladder and pelvic area. Symptoms may also include an urgent need to urinate, a frequent need to urinate, or a combination of these symptoms. Pain may change in intensity as the bladder fills with urine or as it empties. IC/PBS is more common in women than in men. In women, symptoms often get worse during menstruation.

However, because IC varies so much in symptoms and severity, most researchers believe it is not one, but several diseases. In recent years, scientists have started to use the terms bladder pain syndrome (BPS) or painful bladder syndrome (PBS) to describe a set of painful urinary symptoms that may not meet the strictest definition of IC. The term IC/PBS includes all cases of urinary pain that cannot be attributed to other causes, such as infection or urinary stones.

Currently, the cause(s) of IC/PBS remain unknown, and no widely effective treatments are available to treat IC/PBS. Therapeutic approaches are aimed at symptom relief, and include identifying and avoiding factors, such as certain behaviors, foods, or additives, that may trigger symptoms.

**Overlapping Conditions, Multiple Strategies**

At the same time that she was dealing with urinary pain symptoms, Veronica also grew up with another set of symptoms that was diagnosed about 10 years ago as irritable bowel syndrome (IBS). IBS is a functional gastrointestinal (GI) disorder, meaning it is a problem caused by changes in how the GI tract works, but it is not a disease, nor does it cause damage to the GI tract. People with IBS have abdominal pain or discomfort, often reported as cramping, along with diarrhea,

constipation, or both. IBS, like IC/PBS, is much more common in women. Veronica says that, for her, the IBS symptoms are worse in some ways, especially at work, because they are more obvious. "I get gas and bloating and have to use the bathroom. I can hide the IC/PBS symptoms, but not these."

Asked how she has coped with all of these symptoms, especially before she had any diagnoses, Veronica notes that she developed a number of strategies over the years. For the urologic symptoms from IC/PBS, she says, "I'm very careful about what clothes I wear, and wash my clothes and underwear separately from all the other laundry, using fragrance-free detergents," which, she explains, helps with the urinary pain symptoms. She also learned not to hold urine for long periods of time, which she found exacerbated her symptoms. Following her diagnosis, she figured out that other known triggers for IC/PBS symptoms, such as caffeine, were also triggers for her. For urinary pain, she uses over-the-counter medications, such as ibuprofen or acetaminophen.

She also has strategies to deal with the digestive symptoms from her IBS. "I know what foods are triggers," such as fried foods, she says, and is especially careful during the work week. With her doctor's knowledge, she has also recently been following a nearly gluten-free diet. While she has not been diagnosed with celiac disease, an autoimmune disease that damages the digestive tract of affected people when they eat gluten, gluten-containing foods seem to be a trigger for her IBS symptoms. After several weeks on a gluten-free diet, she says that her "stomach is flat (not bloated) now," and she feels much better. Stress is another trigger, and Veronica says she may also look into mindfulness/cognitive behavioral therapy approaches to help with symptom management. However, another part of her personal strategy is to keep things in perspective and try not to get "too crazy" with management—quoting her husband, she says, "everything in moderation, even moderation."

**IC/PBS, Urologic Chronic Pelvic Pain, and the MAPP Research Network**

Veronica's experience is not unique. Researchers, clinicians, and patients alike have been baffled and challenged by IC/PBS. Despite years of committed basic and clinical research efforts, the cause(s) of IC/PBS remain elusive, as do effective treatments. Moreover, a diagnostic test for IC/PBS is not currently available. Instead, because many IC/PBS symptoms can be symptoms of other diseases, those diseases need to be ruled out first—making IC/PBS a "diagnosis of exclusion." While public and clinical awareness of this condition is increasing due to educational efforts by the NIDDK and major health advocacy organizations, such as the Interstitial Cystitis Association, many people still suffer for years with pain symptoms and no diagnosis. Recent data from a major NIDDK-supported epidemiological study suggest that, among adult women in the United States, as many as 2.7 percent have symptoms consistent with IC/PBS. Further, a growing body of evidence suggests that, like Veronica, many people with IC/PBS and other urologic chronic pelvic pain syndromes frequently have other chronic pain conditions, further affecting quality of life.

In 2008, to help better understand the underlying causes of the two most prominent chronic urological pain syndromes, IC/PBS and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), the NIDDK launched the MAPP Research Network. This novel, multi-site research network embraces a unique, systemic (whole-body) approach to the study of IC/PBS and CP/CPPS. In addition to moving beyond traditional bladder- and prostate-specific research

# PATIENT PROFILE

directions, MAPP Research Network scientists are investigating potential relationships between these two urological syndromes and other chronic pain conditions that are sometimes seen in people with IC/PBS and CP/CPPS, such as IBS, fibromyalgia, and chronic fatigue syndrome.

The MAPP Research Network includes scientists with diverse research expertise, all working collaboratively. Clinical researchers bring experience treating patients. Epidemiological investigators study the occurrence of, and identify risk factors for, IC/PBS and CP/CPPS. Basic research scientists examine what is happening on a cellular level. Network leaders include not only urologists, but investigators specializing in the overlapping pain conditions. The MAPP Research Network researchers and staff are assembled at six Discovery Sites that conduct the research studies and two Core Sites that coordinate data collection, analyze tissue samples, and provide technical support.

Currently, the MAPP Research Network is recruiting and characterizing people with IC/PBS and CP/CPPS in a central study to better understand the natural history of these conditions and to see if people with these conditions fall into different, distinguishable subgroups that may suffer from different causes and require different treatments. The Network is also conducting key brain-imaging studies, studies to identify biomarkers of disease, efforts to assess the possible role of infectious agents, and other studies designed to provide a systemic view of disease. Additionally, the Network includes "control" participants—both healthy persons without any pain syndromes, and those who have one or more of the overlapping pain conditions. Through its multi-pronged approach, the MAPP Network aims to discover new and clinically relevant insights that may lead to improved treatment options and better patient care.

*For Veronica, an unexpected but large part of the benefit of being part of the MAPP Research Network has also come from knowing she isn't alone. She says that this is the first time she's been around many people with similar health issues.*

**Participation in the MAPP Research Network**

Following her diagnosis with IC/PBS in January 2012, Veronica said she felt relieved. After so many years, "It was great just to have a diagnosis…to know that there wasn't something 'wrong' with me," she says. Her urologist, who is also a principal investigator for the University of California, Los Angeles (UCLA) site of the MAPP Research Network, then told her about the study and encouraged her to consider joining. "I thought that was great," says Veronica, "anything that can help advance what we know about IC/PBS. Not only that, but at the very least, I thought I could learn a little bit about it from people who've been working on it."

People with IC/PBS or CP/CPPS who enroll in the MAPP Research Network are initially asked to fill out a number of questionnaires covering a variety of topics, including urologic pain, emotional state, other types of pain, and other symptoms and quality of life issues. They also participate in a pressure pain threshold procedure that is another way to assess pain sensitivity, and are asked to provide blood and urine samples for use in some of the research studies. This initial, in-depth clinic-based visit is followed by 2 additional visits at 6 and 12 months after enrollment. During that year, participants are also registered with an internet-based system so that they can fill out assessments of their symptoms every 2 weeks. This ongoing symptom assessment, a key component of the central epidemiological study, is a particularly valuable tool, as it is allowing MAPP Research Network

scientists to learn a great deal about symptoms, long-term symptom fluctuations, and their possible correlations with other factors.

Veronica enrolled in the MAPP study in spring 2012. She says she was intrigued by the questionnaires. She says, "The questions have been wildly interesting… they bring out the detective in me"—especially questions she wasn't initially expecting, such as psychological and emotional questions, or questions about things like sinus pain. Answering the questions "makes (her) think and wonder" about the possible connections. She says that keeping up with the biweekly self-assessments can sometimes be a bit of a challenge with her busy schedule, but she has managed to pretty much stay on top of it.

Many MAPP Research Network participants, including Veronica, also have participated in a brain imaging study that could help scientists better "map" changes associated with chronic pain. Veronica also participated in a special brain imaging study being conducted at the UCLA MAPP Research Network site, focused on using common "stressors," such as cold water, to understand if the brain processes pain differently in people with IC/PBS versus those without the condition.

Veronica also enjoyed being part of one of several special focus groups in which participants have talked about symptom "flares"—*i.e.*, how their symptoms seem to wax and wane. Through these focus groups, the MAPP Research Network scientists are learning a great deal more about flares, their specific nature, why and when they occur, and their short- and long-term impact on individual persons—for example, participants have drawn a distinction between "flares" and "mini-flares." Veronica says she has had flares all her life, but the intensity of flares has changed for the better in recent years—now, she says, she "only gets a bad one once or twice a year," while she may get "mini-flares" five or six times a year. Knowing more about flares will also be helpful to researchers as they consider research studies about IC/PBS and other pain syndromes, as the "flare status" of a participant could potentially affect research results.

**A Family Affair**

When asked what she hopes to see from the MAPP Research Network studies, Veronica says that, while she doesn't expect an immediate result, she does look forward to seeing something down the road, such as an article in the paper saying that researchers from the MAPP Research Network study have made a discovery about IC/PBS. She also wonders generally whether there might be some discoveries about family associations for IC/PBS and overlapping pain conditions. Several members of Veronica's family also have pain syndromes, including IBS, IC/PBS, and fibromyalgia. "If they find that there is a genetic link here, I won't be surprised," she says.

Veronica also can't say enough about how important her family support has been through the years. She and her husband, David, who is also a computer specialist, have been together for 20 years—"We were high school sweethearts," she says happily—and have struggled together through many life challenges, including Veronica's pain and digestive symptoms. "He helps me so much. When he sees me wallowing—which is rare now—he knows it's bad, and does special little things for me." To Veronica, family communications and everyone putting each other first helps immensely in dealing with the challenges of her pain conditions. In her eyes, "You get out what you put in."

Her children are also very supportive. Veronica has explained as much as she can at this point about

# PATIENT PROFILE

what she is going through, trying to be honest without getting into too much detail. They also like that she is participating in a research study. Veronica says, "I try to make it fun," such as by sending them funny photos of herself during a test or when she goes for the acupuncture sessions that help her symptoms. That helps them to ask and talk about it, she says. Veronica says they have also been supportive of new strategies she is trying—for example, they are sharing the gluten-free food she is eating while not totally excluding wheat from their own diets.

Veronica says that her biggest fear is that her daughter may develop IC/PBS symptoms someday. Thus, based on her own experience, she is already introducing routines and strategies that may reduce symptom risk as the "normal" for her daughter, such as separate laundry and no bubble baths. At this point, she says, there have only been a couple of possible episodes, nothing like Veronica's experience as a child.

*Veronica says she would definitely recommend participating in the MAPP Research Network. As Veronica puts it, it is "Reassuring to know that people are working on chronic pain, that something is being done."*

**Perspectives**

Veronica admits that dealing with all of her symptoms has been and remains challenging. Still, her perspective is that, while things are not perfect, her symptoms are better than they were several years ago, she has a diagnosis—which has brought peace of mind—and that, while she would "jump at the chance" not to have to do all the extra work they entail, she has strategies that help her to manage her symptoms. She is also grateful that IC/PBS isn't intrinsically life-threatening, but still happy that scientists are researching it to help people like her who are dealing with chronic pain issues. Most of all, she is determined, especially since having children, not to let her symptoms keep her from life activities. "When you have kids, they have to come first," she says.

As for participating in a research study, for Veronica, an unexpected but large part of the benefit of being part of the MAPP Research Network has also come from knowing she isn't alone. She says that this is the first time she's been around many people with similar health issues, and it has opened up a wealth of opportunities. She has enjoyed getting the opportunity to relate with others and hear their stories, share home remedies and information about food issues and health resources, and even to network. She likes knowing that she is "helping people just like [me]."

Veronica is also very positive about her experience with the MAPP Research Network staff. "The staff have been great—they are very compassionate, show genuine concern, and want to know what is going on with me. I feel safe, and that my confidentiality is assured. I also have access to resources—especially people—I never knew I could. I've had a great experience." Veronica says she would definitely recommend participating in the MAPP Research Network, both for the opportunity to meet other people with similar issues and the opportunity to get peace of mind and know the condition better. As Veronica puts it, it is "reassuring to know that people are working on chronic pain, that something is being done."

*For more information about the MAPP Research Network, see: http://www.mappnetwork.org/*

## ACKNOWLEDGEMENTS

Printed February 2013

### Research

The NIDDK gratefully acknowledges the contributions of the researchers whose studies are described in this report, and of the volunteers who participate in clinical research studies.

### Writing and Production

*Overall Production*

Sandeep Dayal, Ph.D., Office of Scientific Program and Policy Analysis
David Miller, Ph.D., Office of Scientific Program and Policy Analysis

*Highlights of Research Advances and Opportunities*

Staff of the NIDDK Office of Scientific Program and Policy Analysis
Richard Farishian, Ph.D., Director
Lisa Gansheroff, Ph.D., Deputy Director
Rebecca Cerio, Ph.D.
Sandeep Dayal, Ph.D.
Patrick Donohue, Ph.D.
Anne Hanessian
Mary Hanlon, Ph.D.
Eleanor F. Hoff, Ph.D.
David Miller, Ph.D.
Megan Miller, Ph.D.
Dung Pham
Sharon Pope, M.S.
B. Tibor Roberts, Ph.D.
Julie Wallace, Ph.D.
Cheryl Williams, B.S.

Contributions from the NIDDK Office of Communications and Public Liaison
Eileen Newman, M.S., R.D.
Jody Nurik, M.G.A.
Amy F. Reiter, M.S.
Dana Sheets, M.A.

For more information on the NIDDK and its mission, and links to other sites, visit: *www.niddk.nih.gov*

Scan the QR code with your mobile device to link to the NIDDK web-site at *www.niddk.nih.gov*

National Institute of Diabetes and Digestive and Kidney Diseases

NIH Publication Number: 13-7962